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# Review of Laboratory Program on Degradation Mechanisms in Soil of Wastewater from Nitroguanidine Manufacture

**FINAL REPORT**

**March 1987**

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**Prepared for:**
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The degradation of nitroguanidine manufacture wastewater components was examined in order to predict the long-term feasibility of land farming. Continuous flow and soil perfusion columns, microbial enumeration, and batch mineralization studies were utilized for this investigation.

After 271 days of operation for the continuous flow soil columns and 84 days of operation for the soil perfusion columns, only some components of nitroguanidine wastewater were completely or partially removed. Guanidine nitrate and sulfate were the most rapidly transformed. Nitroguanidine (NQ), however, was only partially removed.

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19. ABSTRACT (continued)

Enumeration of the SFAAP soil before and after wastewater treatment indicated no increase in the number of soil microorganisms able to degrade nitroguanidine after 271 days exposure. In addition, no significant change was observed in the total soil microbial population size. The composition of these populations was not evaluated.

In mineralization studies, guanidine nitrate was readily transformed, with greater than 50 percent of the parent compound evolved as $CO_2$. Nitroguanidine, however, was poorly transformed, with less than 25 percent of the parent compound evolved as $CO_2$. Addition of carbon supplements, nutrients, acclimated microorganisms, or incubation under aerobic or anaerobic conditions did not significantly alter mineralization of the two compounds.

This study indicates that nitroguanidine would probably be poorly removed in a land treatment system, and could potentially contaminate groundwater. In addition, inorganic constituents of wastewater, such as nitrate and sulfate, could contaminate groundwater.

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# Review of Laboratory Program on Degradation Mechanisms in Soil of Wastewater from Nitroguanidine Manufacture

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# EXECUTIVE SUMMARY

A complete review of the literature on biodegradation of nitroguanidine wastewater components indicated that insufficient data existed to predict the long-term feasibility of land farming nitroguanidine manufacture wastewater. This report reviews a laboratory program conducted by WESTON for USATHAMA on degradation mechanisms in soil of simulated nitroguanidine manufacture wastewater.

Experimentation included three major areas of investigation: Continuous flow and perfusion soil columns receiving simulated nitroguanidine wastewater; enumeration of Sunflower Army Ammunition Plant (SFAAP) soil in order to detect possible microbial adaptation; and batch mineralization studies using isotopic nitroguanidine and guanidine nitrate in SFAAP soil.

After 271 days of operation for the continuous flow soil columns and 84 days of operation for the soil perfusion columns, only some components of nitroguanidine wastewater were completely or partially removed. Guanidine nitrate and sulfate were the most readily transformed. Nitroguanidine (NQ), however, was only partially removed. Carbon supplements did not significantly enhance guanidine nitrate degradation, but sulfate and NQ removal was facilitated by supplemental carbon.

Sorption of nitroguanidine to soil particles was observed. The capacity for, and stability of, sorption of NQ to soil was not studied. Sorption was observed in our short-term soil mobility test and limited NQ accumulation was detected within the continuous flow soil columns.

Enumeration of the SFAAP soil before and after wastewater treatment indicated no increase in the number of soil microorganisms able to degrade nitroguanidine after 271 days exposure. In addition, no significant change was observed in the total soil microbial population size. The composition of these populations was not evaluated.

In mineralization studies, guanidine nitrate was readily transformed, with greater than 50 percent of the parent compound evolved as $CO_2$. Nitroguanidine, however, was poorly transformed, with less than 15 percent of the parent compound evolved as $CO_2$. Addition of carbon supplements, nutrients, acclimated microorganisms, or incubation under aerobic or anaerobic conditions did not significantly alter mineralization of the two compounds.

This study indicates that nitroguanidine would probably be poorly removed in a land treatment system, and could potentially contaminate groundwater. In addition, inorganic constituents of NQ wastewater, such as nitrate and sulfate, could contaminate groundwater. Lagooning of nitroguanidine wastewaters under conditions that encourage biological denitrification could be a potential solution to the nitrate problem. It is advisable, however, that NQ be treated (for example, by the method currently used) prior to introduction of wastewater to a biological treatment system.

# 1. INTRODUCTION

1.1 Background. Nitroguanidine (NQ) is a water soluble nitroamino compound used as an ingredient in munitions propellants. It is produced at the Sunflower Army Ammunition Plant (SFAAP), DeSoto, Kansas. A number of waste streams are produced during NQ manufacture, and these wastewaters may contain NQ (0.4 - 2,500 mg/l), guanidine nitrate (1.0 - 200 mg/l), ammonia (1.0 - 200 mg/l), nitrate (1.0 - 11,800 mg/l), and sulfate (0 - 5,500 mg/l).

If left untreated, this wastewater has the potential to cause environmental disruption and toxic effects. Currently, wastewater is treated with lime to pH 11 to 12 and then sparged with steam to degrade the NQ. This water is then stored in lagoons. Activated carbon and ion exchange are being tested as alternative methods for wastewater treatment. Land farming has also been proposed as a means of treating these materials. In order for land farming to be an acceptable treatment technology, components of the wastewater must be biodegradable or subject to other fate processes such that only innocuous products remain in the environment.

Previous studies have investigated the biodegradability of nitroguanidine in aqueous systems and soil. Attempts to demonstrate the biological mineralization of NQ in aqueous systems have failed (1). Although NQ was reduced to nitrosoguanidine in anaerobic continuous culture, no transformation of NQ occurred under aerobic conditions. Later studies indicated that nitrosoguanidine was not biologically degraded but did decompose nonbiologically to a number of transformation products. NQ has been found to biodegrade in soil, but a carbon supplement is required. During this cometabolic biodegradation, 85 percent of the nitrogen added as NQ was converted to ammonia. No significant concentration of potentially hazardous organic intermediates was detected.

NQ wastewater has been stored in lagoons at SFAAP and continues to accumulate. Disposal of this wastewater by land treatment has been attempted, but final disposition of components has not been adequately measured. Application rate was based on nitrogen loading relative to natural utilization processes (2). The impact of the wastewater on the soil-plant system within the application site was considered to be negligible (3). This land treatment was inconclusive regarding the feasibility of continued land farming for two reasons. First, the test concentration was not representative of a NQ production waste stream. Second, the test site was fertilized during the test with nitrogen, thereby making the results difficult to interpret.

1.2 Project objective. The objective of this study is to develop sufficient data to either recommend or not recommend land application. The objective requires determining whether or not the components of NQ wastewater are transformed to innocuous products under circumstances environmentally relevant to SFAAP. Continuous flow soil columns and soil perfusion columns receiving simulated NQ wastewater have been studied. The rate of degradation of NQ and GN in SFAAP soil before and after wastewater application has been determined using $^{14}C$-labeled compounds. The affect of nutrients, aerobic and anaerobic conditions, and cometabolic substrates on degradation of NQ and GN was investigated. In addition, the number of microorganisms able to degrade NQ and GN in SFAAP soil was evaluated before and after simulated wastewater treatment.

This report is organized according to the following format: Introduction; Literature Review; Materials and Methods; Results; Discussion; and Conclusions and Recommendations. Only illustrative data are presented in the text. All data compiled during the study are presented in the report appendices.

## 2. LITERATURE REVIEW

2.1 Environmental setting. The Sunflower Army Ammunition Plant (SFAAP) is located in the Osage section of the Central Lowlands Province between two tributaries of the Kansas River. It consists of approximately 9,000 upland acres that are generally well drained and rarely subject to flooding. Depth to the groundwater saturated region is greater than 20 feet in most areas. SFAAP is underlain with nearly horizontal beds of limestone and shale. The soil depth above bedrock ranges from 12.3 to 44 feet. A firm clay subsurface layer approximately 15 to 30 inches thick is located 12 to 18 inches below the soil surface. Permeability is moderate, ranging from $6.7 \times 10^{-3}$ cm/sec (9.54 in./hr) to $2.3 \times 10^{-5}$ cm/sec (0.03 in./hr) (3).

Nutrients (organic nitrogen, ammonia-nitrogen, nitrate/nitrite-nitrogen, potassium, and phosphorus) in SFAAP soils are generally present at very low levels (2). The amount of organic carbon in the A horizon was found to exceed 2 percent in most cases. In addition, the soils were fairly acidic (pH 6.1), indicating the potential for heavy metals migration, but also had a very high cation exchange capacity.

Groundwater in the SFAAP area did not exceed primary or secondary drinking water standards (2). Fluoride and nitrate were closest to exceeding these standards. Fluoride was present at 0.33 to 0.80 mg/l and nitrite/nitrate-nitrogen at 2.6 to 4.0 mg/l.

2.2 Fate of nitroguanidine in aqueous systems. Attempts to demonstrate the biological mineralization of NQ in aqueous systems have failed (1). NQ was cometabolically reduced to nitrosoguanidine in anaerobic continuous culture using acclimated microorganisms. Media utilized in the anaerobic chemostats were either nutrient broth (2.4 and 8 g/l), basal salts, basal salts with glucose, or basal salts with glucose and nitrogen, all of which were incubated at 37°C. Digest from a municipal sewerage treatment plant was acclimated for use as the anaerobic inoculum. NQ was not detectable in the anaerobic culture vessel after seven days, and none was present in the effluent reservoir after 12 days. During this time nitroso-guanidine accumulated in the expended media. No transformation of NQ occurred under aerobic conditions (1).

In response to the accumulation of the potentially harmful nitrosoguanidine, further studies were conducted. These studies confirmed that nitrosoguanidine was not biologically degraded under aerobic or anaerobic conditions in aqueous systems (4).

Nitrosoguanidine was, however, nonbiologically decomposed during aerobic incubation at 30°C and anaerobic incubation at 37°C (4). The rates of disappearance were higher at the higher incubation temperature. The chemically unstable nitrosoguanidine was transformed into cyanamide, cyanoguanidine, melamine, guanidine, and nitrosamide. The nitrosamide further decomposed to nitrogen gas and water (1).

In a recent study conducted by Polybac Corporation (5), initial microbial toxicity testing on NQ manufacture wastewater concluded that the raw acidic wastewater was toxic to biological populations. However, the solar pond (lagoon) water, pre-treated with lime and heated to 70°C, was not toxic to microbial populations. A biological submerged film reactor system was used to treat the SFAAP solar pond water. The film consisted of a rigid PVC matrix that promoted bacterial population attachment. The film was used in five connected chambers under varied incubation conditions (three anaerobic and two aerobic) to promote organic biodegradation, denitrification, deammonification, sulfate reduction, and other biological reactions to transform nitroguanidine manufacture wastewater to innocuous products. Nitroguanidine, guanidine nitrate, ammonia, nitrate, nitrite, and COD levels were decreased by the system, but NQ was only partially transformed. The overall average removal of NQ was 30 percent. No attempt was made to distinguish biological from abiological degradation.

Both NQ and nitrosoguanidine were sensitive to UV light. The photolytic and chemical pathways for the decomposition of nitrosoguanidine apparently result in similar products (1).

2.3 Nitroguanidine and metabolites in soil. The first reported study of the biodegradation of nitroguanidine was conducted by the American Cyanamid Company in 1955 (6). Nitroguanidine was mixed with dry soil (200 g), comprising from 2 percent to 0.2 percent of the total soil mass, and then wetted with BOD dilution water. Mineralization of nitroguanidine was determined by measuring evolved $CO_2$ or ammonia. The test was run for two weeks. Results were ambiguous due to overlap in $CO_2$ production concentration between the controls and test samples.

Studies have also been conducted on biodegradation of NQ in continuous flow soil columns packed with garden soil (6.5 percent organic matter, pH 6.9) (7). The soil was inoculated with organisms from a mixture of activated sludge, anaerobic sludge digest, and garden soil. In soil supplemented with glucose as an alternate carbon source, NQ was degraded to ammonia. Cometabolism was essential for the biodegradation of

NQ in soil. Glucose at 1.0 to 0.5 percent, equivalent to C/N ratios of 68 to 1 and 34 to 1, was required for biodegradation of NQ to ammonia (7). No significant concentrations of potentially hazardous organic intermediates were detected.

Ammonia in the soil column effluent accounted for 85 percent of the nitrogen added to the column as NQ. Throughout the study the level of ammonia in the column leachates correlated with NQ degradation (7).

Nitrate and nitrite remained at background levels in both influent and effluent. Initial levels of nitrate in the effluent were as high as 80 mg/l but dropped off progressively with time. Nitrite in the effluent generally remained in the µg/l (ppb) range. Periodic fluctuations as high as 3 mg/l were noted in the effluent but corresponded to nitrite fluctuations in the influent.

Guanidine and organoguanidine, both products of nonbiological decomposition of nitrosoguanidine, have been found to be degraded by soil bacteria (8). Using a soil perfusion laboratory method, soil bacteria degraded guanidine and organoguanidine under anaerobic conditions. Activated sludge, however, did not degrade these compounds.

Kaplan and Kaplan (7) extended the results of their laboratory study using garden soil to a land treatment system. They determined land application rates based on the surface area (44.2 $cm^2$) of their soil columns and the feed rates used (approximately 100 ml/day). They postulated that an in situ application rate of approximately 22,700 gallons/day (86,000 liters/day) per acre could be utilized. This would result in 20 pounds (9.1 kg) of NQ/day/acre.

Kaplan and Kaplan recommended a land treatment monitoring system be set up to detect the products of NQ degradation in soil. For short-term land application systems, they suggested monitoring, at a minimum, NQ, nitrosoguanidine, nitrates, nitrites, ammonia, total organic carbon, and total nitrogen in process water, groundwater, and soils.

2.4 Land farming SFAAP. Five million gallons of prove-out wastewater generated from the "Calciner" facility at SFAAP had accumulated in two storage basins by the fall of 1983. A decision was made to dispose of this wastewater by land application. Boldt et al (2) based their one-time application system on nitrogen loading. Consequently, ammonia and nitrate levels were the parameters of concern. Wastewater components such as heavy metals were considered to be at an insignificant

level. The critical assumption for their system was that all the nitrogen being applied would be either volatilized, nitrified, denitrified, or taken up by plants such that the nitrogen content of groundwater, surface water, and soil would not reach harmful levels.

The concentration of NQ actually in this stored wastewater at the time of application was quite low. The application waste stream was above 0.1 mg/l NQ on only three successive days. The impact of the wastewater on the soil-plant system within the application site was considered to be negligible (3). This study is inconclusive regarding the feasibility of NQ land farming because the test concentration was not representative of a NQ production waste stream.

In addition to the problem of low NQ content, the test site was fertilized during the test with 36 pounds of nitrogen per acre (3). Therefore, the source of the high nitrogen concentrations detected in these soils is uncertain. All nitrogen forms did increase during the study. Nitrite/nitrate in the surface and subsurface soils increased anywhere from 50 to 100 percent. Ammonia-nitrogen was 5 to 10 times higher than values recorded for the same soil in 1982. In general, organic nitrogen increased between two and three times. Total nitrogen in December 1982 ranged from 1,200 to 1,800 µg/g for surface soils and from 300 to 800 µg/g for subsurface soils. After the NQ application and fertilization, total nitrogen ranged from 2,000 to 3,400 µg/g for subsurface samples.

NQ and guanidine nitrate in soil samples from the SFAAP application site were below the detection limit (3). Soil phosphate, fluoride, and pH were not greatly effected by the wastewater land application. Several parameters, however, were changed by the wastewater application. The cation exchange capacity (CEC) of the surface and subsurface soils decreased slightly. Prior to treatment, the CEC of the surface soils ranged from 20 to 40 meq/100 grams of soil; the subsurface soils ranged from 36 to 40 meq/100 grams of soil. After treatment, the CEC decreased to 20 to 30 meq/100 grams for both the surface and subsurface soils.

In general, there was an overall reduction in the carbon content of both surface and subsurface soils following wastewater application. The exact decrease cannot be determined because of the analytical procedures utilized. The initial samples were analyzed for total organic matter. The post-treatment samples were analyzed for total organic carbon (TOC).

2.5 NQ migration in soil. The fate and transport of NQ in soil can be affected by factors such as mobility, adsorption, and diffusion. NQ is a very mobile soil contaminant. The adsorption coefficients that have been measured are less than 2 for all soils except those with high organic carbon. Organic carbon and clay content significantly affect NQ adsorption. Of the two, organic carbon has a much stronger effect. Experimental results indicate that adsorption is an important fate process for NQ when organic carbon content exceeds 3 percent (9). NQ has a low volatility and is expected to remain in soil. Diffusion of NQ is an insignificant transport process except in soils having more than 30 percent clay and percolation rates less than 1 inch/year (9).

2.6 Toxicology. NQ and nitrosoguanidine gave negative results in the Ames screening test for mutagenicity (Kaplan et al, preprint). NQ, however, was reported to be a carcinogen in screening tests with Chinese hamster cells (10).

It should be noted that N-methyl-N-nitro-N-nitrosoguanidine is both a mutagen and carcinogen (10). Nitrosoguanidine is a suspected carcinogen, but insufficient information is available to assess its exact risk. Improper use of nomenclature has created confusion in the literature regarding the distinction between these two compounds (11).

2.7 Structural Formulas. The structural formula of key nitroguanidine manufacture wastewater components and degradation intermediates are shown in Figure 2-1.

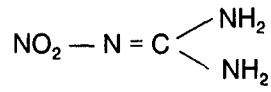
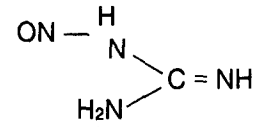
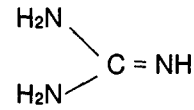
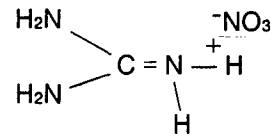
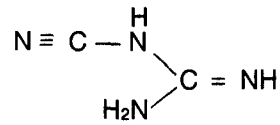
| **Name** | Abbreviation | Structural Formula |
|---|---|---|
| Nitroguanidine | (NQ) |  |
| Nitrosoguanidine | (NOQ) |  |
| Guanidine | (G) |  |
| Guanidine Nitrate | (GN) |  |
| Cyanamide | (CY) | $H_2NC \equiv N$ |
| Nitrate | ($NO_3$) | $NO_3$ — |
| Nitrite | ($NO_2$) | $NO_2$ — |
| Ammonia | ($NH_3$) | $NH_3$ |
| Cyanoguanidine | (CG) |  |

**Figure 2-1. Structural formulas.**

# 3. MATERIALS AND METHODS

## 3.1 Soil handling and characterization.

3.1.1 Collection. Surface soil (upper 18 inches) was collected at the Sunflower Army Ammunition Plant (SFAAP), Kansas. Soil was obtained from an area adjacent to the site utilized for the one-time application of NQ prove-out wastewater (see Section 2, Literature Review). Soil was collected using a shovel and new five-gallon metal pails. Sod and larger stones were excluded from the sampling. Soil was shipped to WESTON immediately after collection.

3.1.2 Storage. SFAAP soil was stored in glass jars. The jars were filled 3/4 full, covered with plastic wrap, and sealed with a lid. Soil was stored in the dark at 4°C in a solvent-free, walk-in refrigerator.

3.1.3 Preparation. Soil was prepared for column studies by sieving between U.S. Standard Sieves #1 (6.3 mm/0.250 in. openings) and #5 (4.0 mm/0.157 in. openings). Only those soil particles which remained between the two sieves were used. In order to facilitate sieving, the soil was air dried for approximately 16 hours. Soil was prepared for mineralization studies by macerating it into small particles with a stainless steel spatula before adding it to mineralization flasks.

3.1.4 Sterilization. Sterile soil controls were heat killed (121°C, 15 psi for 30 minutes on three consecutive days) or inactivated by the addition of 0.75 percent mercuric chloride. Controls for $^{14}$C-MPN enumerations were heat sterilized. Continuous flow soil column controls were inactivated using 0.75 percent mercuric chloride in the feed solution. Rate of mineralization controls were heat sterilized and mercuric chloride inactivated. Sterility was verified by taking a 0.5 g sample of soil and adding it to a culture tube containing nutrient broth. Observations for turbidity were made after 48 hours incubation at 35°C. Bacterial enumerations were done at two-week intervals on the soil column effluents using standard plate count methods (12).

3.1.5 Inoculation.

3.1.5.1 Inoculum. Soil in perfusion columns and continuous flow columns was inoculated with a mixture of activated sludge (Avondale Sewage Treatment Plant, Avondale, Pennsylvania), anaerobic sludge digest (Ocean County, New Jersey), and extract from garden soil, (West Chester, Pennsylvania). A mixture containing equal portions of these was diluted with 0.085 percent potassium chloride. The 30 percent solution was filtered and the filtrate used as the inoculum. Mineralization study soil was inoculated with activated sludge or extract of acclimated soil.

3.1.5.2 Establishment of inoculum source (acclimated soil). A small glass column (100 mm x 70 mm) was packed with 200 g of SFAAP soil. The top of this column was seeded with a total of 25 g of soil taken from each of the active continuous flow soil columns. These soil columns had been receiving NQ wastewater for approximately 20 days. An influent stream of NQ wastewater with carbon supplements (whey, molasses, and glucose) was maintained at approximately 4 ml/hour. The effluent stream was discarded.

3.1.5.3 Harvesting inoculum. After a minimum run time of 60 days, the column was separated from influent and effluent lines. 100 g of soil was removed from the influent end of the column. The column then was repacked with 100 g of fresh SFAAP soil, inverted, and reattached to influent/effluent lines. Soil removed from the column was water extracted, and the extract was used as an inoculum.

3.1.6 Extraction of microorganisms from soil. Microorganisms were extracted from soil in 90 ml of filter sterilized ammonium phosphate buffer (0.1M $NH_4H_2PO_4$). Five drops of Tween 80 (Polyoxyethylene sorbitan monoleate) were added to the soil and buffer solution. The 125 ml flask was stoppered and agitated on a wrist-action shaker at 1/2 maximum speed for 20 minutes. Only the coarsest sand particles were allowed to settle before using the solution for inoculation or enumeration.

3.1.7 Soil pH. Soil pH was determined by placing 20 g of soil in a 50 ml beaker, adding 20 ml of distilled water, and stirring the suspension several times during the following 30 minutes. The soil suspension was allowed to stand for 1 hour and the pH of the liquid determined by electrodes (13).

3.1.8 Soil moisture. A clean, dry container and lid were weighed prior to the addition of approximately 20.0 g of soil. The lid was immediately replaced and the container weighed. The lid was then removed, and the container with the moist soil sample was placed in a drying oven maintained at 230 ±9°F until a constant weight was reached. Immediately upon removal from the oven, the lid was replaced and the sample allowed to cool to room temperature. Percent soil moisture was calculated as follows:

Moisture Content = [(weight of moisture)/weight of oven-dried soil)] x 100 (14).

3.1.9 Water-holding capacity. A small piece of filter paper was folded, placed in the neck of a glass funnel, and moistened. A 10 g soil sample was weighed and placed in the lined funnel. Water was added to the soil by pipette and the amount necessary to saturate the sample noted. The soil was moistened slowly until the first movement of the free-water line was observed in the funnel. The soil was then placed on a preweighed aluminum tray and dried overnight at 100°C. The tray and dried soil were reweighed and the following equation was used to calculate water-holding capacity per gram of soil:

Water added + (weight of original soil sample - dry weight)/dry weight

3.1.10 Total organic carbon in soil. Ten grams of sieved soil was pretreated with 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of concentrated $H_2SO_4$. The suspension was mixed and allowed to stand for 30 minutes. Two hundred milliliters of water were added to the flask and the suspension was filtered. Three to four drops of O-phenanthroline indicator were added, and the solution titrated with 0.5 N $FeSO_4$. The following equation was used to calculate TOC with a correction factor, f = 1.33 (13):

% organic carbon = (milliequivalents $K_2Cr_2O_7$ - milliequivalents $FeSO_4$) x 0.003 x 100/grams water-free soil x (f).

3.2 Analytical. Analytical methods are briefly described in this subsection and listed in greater detail in Appendix A.

(a) Nitroguanidine, nitrosoguanidine and cyanoguanidine - HPLC with a Zorbax $C_8$ reverse phase column, DI water mobile phase and UV detection at 235 nm. Method detection limits were approximately 100 µg/l for nitroguanidine, approximately 200 µg/l for nitrosoguanidine, and approximately 200 µg/l for cyanoguanidine.

(b) Melamine - HPLC with a Zorbax $C_8$ reverse phase column and a 28 percent methanol in 0.005 M octanesulfonic acid solution adjusted to pH 3 with acetic acid. Effluent was monitored at 235 nm and the tested detection limit was approximately 30 µg/l.

(c) Guanidine - Ion chromatography using a cation exchange column with a cation concentration precolumn. The eluent was 0.25 mM hydrochloric acid. Detection limit for guanidine by ion chromatography was approximately 500 µg/l.

(d) Cyanamide - Spectrophotometric determination after complexation with pentacyanoamine ferrate reagent. Detection limit was approximately 100 µg/l.
(e) Guanidine nitrate - Qualitatively determined by thin layer chromatography (TLC) using cellulose plastic backed plates in a butanol/ethylacetate/water (4/1/1) system.
(f) Ammonia-nitrogen - Potentiometric determination using an ion selective ammonia electrode and a pH meter.
(g) Nitrate and nitrite - Determined by ion chromatography.

3.3 Isotopes and scintillation.

3.3.1 Isotopes. The $^{14}C$-labeled substrates used included an amino acid mixture (U-$^{14}C$, Specific Activity 25 m Ci/mg atom); glucose (U-$^{14}C$, Specific Activity 50-60 m Ci/m mol) obtained from Amersham, Arlington Heights, Illinois; guanidine nitrate (U-$^{14}C$, Specific Activity 5-10 m Ci/m mol); and nitroguanidine (U-$^{14}C$, Specific Activity 5-10 m Ci/m mol) obtained from Pathfinder Laboratories, Inc., St. Louis, Missouri. Chemical and radiochemical purity were determined by the manufacturers using thin layer chromatography and liquid chromatography. Radioassay was accomplished via liquid scintillation counting by both the manufacturer and WESTON. $^{14}C$-labeled substrates were diluted with water to prepare stock solutions: amino acids, $1.10 \times 10^6$ dpm/ml; glucose, $1.08 \times 10^5$ dpm/ml; guanidine nitrate, $2.37 \times 10^6$ dpm/ml; and nitroguanidine, $2.08 \times 10^6$ dpm/ml.

3.3.2 Trapping solutions, scintillation cocktails, and counting. Mineralization flasks were purged using positive or negative pressure with air or nitrogen at a rate of 33 ml/minute. (See sections on mineralization apparatus and operations for a detailed description of set-up.) $^{14}CO_2$ evolved from degradation of nonvolatile $^{14}C$-substrates was trapped in a solution containing a 1:7 ratio of mono-ethanolamine and methoxyethanol. Scintillation cocktail used was PCS® (Amersham Corp.). $^{14}CO_2$ evolved in the volatile compound mineralization apparatus was trapped and counted in Oxasol (National Diagnostics, Somerville, New Jersey). $^{14}C$-volatile organics were trapped and counted in Betafluor (National Diagnostics).

Aqueous effluent samples from soil mobility columns were counted in Aquasol-2 (Dupont, NEN Research Products).

$^{14}C$ was counted in a Tracor Analytical 6895 Liquid Scintillation counter. Three background samples (no $^{14}C$ present) were included with each batch of samples run. The scintillation counter was preprogrammed to read background counts first, average them, and subtract the result from each subsequent sample. The Tracor Analytic 6895 computer system converted cpm to dpm. Scintillation vials were counted for 2 minutes each.

3.3.3 Mass balance of radioisotope studies.

3.3.3.1 Acidification of soil. At the conclusion of a mineralization experiment, a final $^{14}CO_2$ determination was performed. The test was terminated by the addition of 25 ml of 1.0 N HCl. Flasks were purged to trap $^{14}C$-carbonates released as $^{14}CO_2$ from the soil after acidification.

The acidified soil slurry was centrifuged at 10,000 G for 30 minutes. The clarified supernatant was decanted into a clean graduate cylinder and the total volume was recorded. A 1 ml subsample of the supernatant was added to 20 ml of 3A toluene cocktail (1:1 mix of PCS® and reagent alcohol) and counted.

3.3.3.2 Soil Combustion. The centrifuge tube (from 3.3.3.2) containing the soil pellet was weighed to determine the quantity of wet soil, and three 0.5 g samples of the soil were burned in a combustion furnace (800°C). The evolved $^{14}CO_2$ was independently collected for each sample in 50 ml of trapping solution, monoethanol amine/methoxyethanol. A 10 ml volume of the trapping solution was added to 10 ml of cocktail solution, and PCS® and counted. The resulting counts for the supernatant and soil burns were corrected for the total volume and weight of the respective samples.

After appropriate volume and weight corrections were performed, the mass balance of radioactivity ($^{14}CO_2$, $^{14}C$-carbonates, $^{14}C$-incorporated in soil, and soluble $^{14}C$-activities) was determined.

3.3.3.3 Calibration/recovery procedure. The efficiency (percent recovery) of the combustion system was determined by comparing the $^{14}CO_2$ activity of the trapping solution after the combustion of a spiked sample to the activity of the trapping solution directly spiked with an equivalent amount of the sample radiolabeled material. When the combustion furnace was not in operation for several weeks or more, a series of standards (spiked samples) were prepared to check recovery and linearity of response (calibration). On an ongoing basis, at least one standard was included with each daily batch of samples. Daily standards were within 90 percent of the expected value.

3.4 Simulated wastewater, solutions, and supplemental media.

Simulated wastewater contains in milligrams per liter of deionized water: nitroguanidine 129; guanidine nitrate 10.50; ammonia nitrate 12.50; and sodium sulfate 166.25. The wastewater was maintained in influent reservoirs under sterile conditions for soil column studies.

Nutrient solution contains in 100 ml of deionized water: yeast extract 0.10 g; trace metal solution 10 ml; and phosphate buffer 10 ml.

Trace metals solution contains in grams per liter of deionized water: nitrilotriacetic acid 1.00; $MgSO_4H_2O$, 2.00; $FeSO_47H_2O$, 0.12; $MnSO_47H_2O$, 0.03; $ZnSO_4 \cdot 7H_2O$, 0.03; and $CaSO_4$, 0.01.

Phosphate buffer plus ammonia contains in grams per liter of deionized water: $K_2HPO_43H_2O$, 42.50; $NaH_2PO_4 \cdot H_2O$, 10.00; $NH_4Cl$, 20.00.

Total plate count agar contains per 200 ml of deionized water: nutrient broth 2.00 g; purified agar 4.00 g; trace metals solution 25 ml; and phosphate buffer 25 ml.

Nitroguanidine enumeration medium contains per 200 ml of deionized water: nitroguanidine 0.04 g; purified agar 4.00 g; trace metal solution 25 ml; and phosphate buffer 25 ml. (For guanidine nitrate enumeration medium, replace NQ with 0.004 g of GN)

Nitroguanidine cometabolism medium contains per 200 ml of deionized water: purified agar 4.00 g; nitroguanidine 0.04 g; dextrose 2.50 g; trace metals buffer 25 ml; and phosphate buffer 25 ml. (For whey cometabolism, replace dextrose with whey)

Anaerobic indicator medium contains per liter of deionized water: 4 ml of 0.05% resazurin; and 15.00 g of purified agar. Heat to a boil, while being stirred with a magnetic stir bar and sparged with nitrogen. Color change from blue to pink indicates presence of oxygen.

Carbon supplements:

Molasses contains: 3.00 percent protein; 1.00 percent nitrogen; 46.00 percent sugar; 10.00 percent ash; 0.50 percent calcium; 0.05 percent phosphorus; and 3.60 percent potassium (Personal communication, Nancy Finkelstein, Cargill Company).

Dry sweet whey contains in mg/100 g: potassium 2080.00; phosphorus 932.00; sodium 1079.00; magnesium 176.00; calcium 796.00; iron 0.88, and zinc 1.97. Dry sweet whey contains 13.10 percent protein and 0.50 percent nonprotein nitrogen (Personal communication, Fred Pepper, Whey Products Institute).

Glucose contains in g/mole: hydrogen 12; carbon 72; and oxygen 96.

3.5 Continuous flow soil columns.

3.5.1 Apparatus. Continuous flow soil columns were 70 mm O.D. x 400 mm long glass columns sealed at each end by a one-hole rubber stopper. A pore stone was used to retain the soil in the column and disperse the influent stream at the top of the column. One kg of soil was added to each column and packed using a manually operated metal rammer. Soil was packed to minimize air space and was monitored for channeling of the influent stream. The column, collection flasks, and reservoirs were covered with aluminum foil to prevent photodegradation and growth of photosynthetic organisms (Figure 3-1).

A manostat peristaltic pump with multichannel cassettes (capable of delivering 0.25 to 500 ml/hr) was utilized to deliver 4 ml of simulated wastewater per hour. The feed solution was pumped through silicone rubber tubing onto the tops of the columns. The solution leached through the soil via gravity.

3.5.2 Operations. Simulated land treatment of nitroguanidine wastewater consisted of six continuous flow columns containing SFAAP soil. Column 1 was treated with deionized water; active microorganisms were present and no carbon supplement was added. Column 2 was treated with wastewater; active microorganisms were present and no carbon supplement was added. Column 3 was treated with wastewater; the soil was sterilized, and supplemented with 1.0-2.0 percent glucose. Column 4 was treated with wastewater; active microorganisms were present and provided with 1.0-2.0 percent glucose. Column 5 was treated with wastewater; the soil was sterilized, and supplemented with 1.0-2.0 percent sweet whey. Column 6 was treated with wastewater; active microorganisms were present and provided with 1.0-2.0 percent sweet whey.

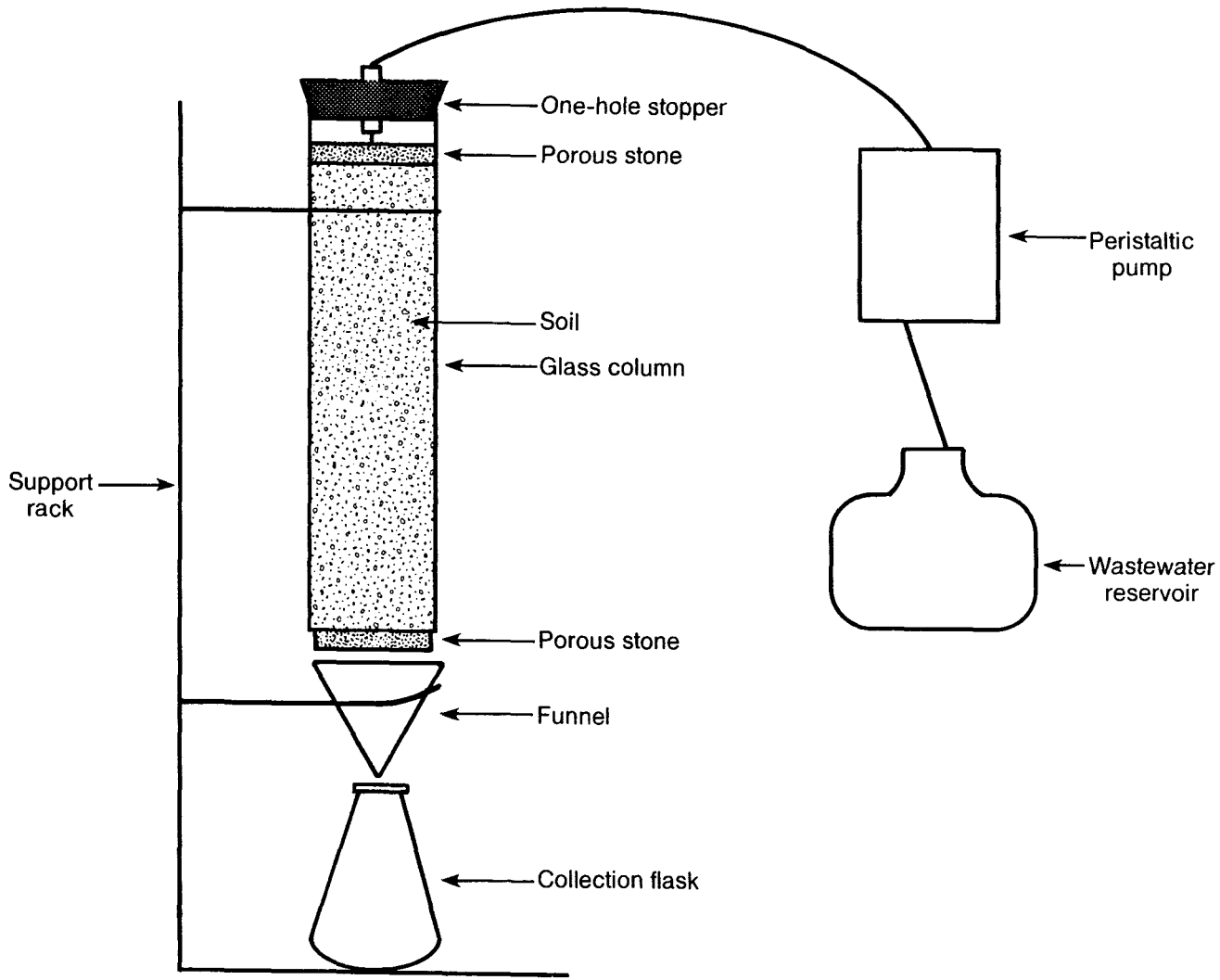



**Figure 3.1. Continuous flow soil column**

3.5.2.1 Sampling of continuous flow soil columns. Influent and effluent for each column were sampled and analyzed for nitroguanidine, nitrosoguanidine, guanidine nitrate, guanidine, cyanamide, melamine, cyanoguanidine, ammonia, nitrite-nitrate, sulfate, and total organic carbon (TOC). The effluent from the columns was collected in Erlenmeyer flasks. Effluent in the flasks was collected each day. Samples were processed as rapidly as possible. During all holding times, samples were stored in the dark at 4°C.

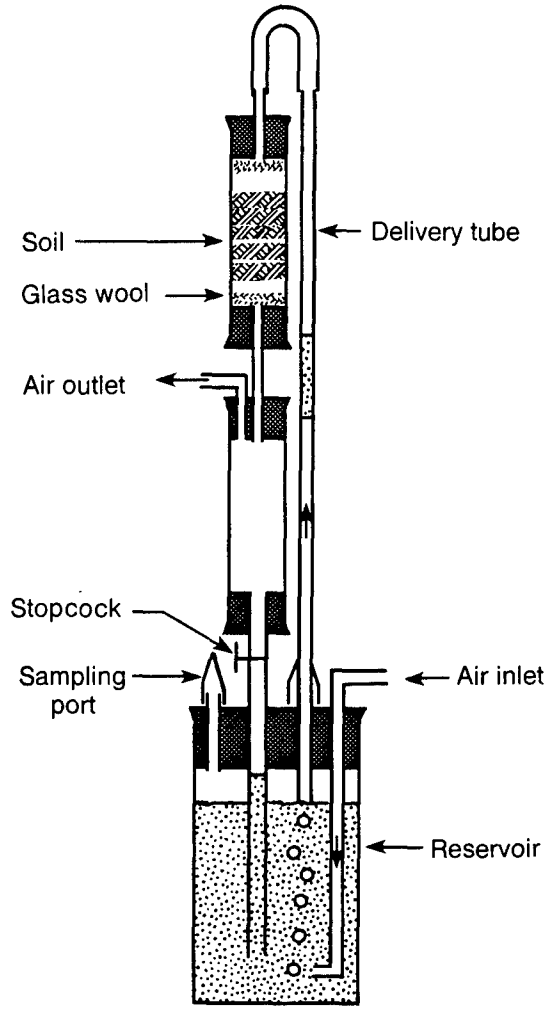
3.5.2.2 Viability of soil columns. Influent and effluent samples from each column were tested for sterility every two weeks using standard plate count methods.

3.5.3 Calculation of Nitrogen Mass Balance. The following steps were followed:

- Start with mg/l of compound for each sample and multiply by volume of sample to yield mg of compound per sample.
- Add all mg of compound per sample values to obtain total mg of compound in influent or effluent.
- Calculate totals for influent and effluent samples separately.
- Determine percent of compound molecular weight which is nitrogen.
- Multiply total mg of compound by percent nitrogen in compound to get total mg nitrogen from each compound.
- Theoretically, total mg of nitrogen in effluent and post-treatment soil should equal total mg of nitrogen in original soil and influent.
- Because nitrate and nitrite concentrations were determined as one value, nitrogen mass balance for the two compounds was calculated as one value. The average calculations assume that the two compounds always occur in a 1:1 ratio. Although this is not the case, it permits a close approximation of actual levels based on available information.

3.6 Perfusion columns.

3.6.1 Apparatus. Soil perfusion columns were positive pressure systems based on a unit described by Kaufman (15). The soil containing columns were enlarged to 70 mm O. D. x 400 mm long with a 70 mm O. D. x 150 mm long trap separating the soil column and 100 mm O. D. x 175 mm high wastewater reservoir (Figure 3-2).




Modified from Kaufman, 1965

**Figure 3.2. Soil perfusion unit.**

3.6.2 Operations. Four soil perfusion columns were filled with SFAAP soil. Column 1 was treated with deionized water; active microorganisms were present and no carbon supplement was added. Column 2 was treated with wastewater; active microorganisms were present and no carbon supplement was added. Column 3 was treated with wastewater; the soil was sterilized, and supplemented with sweet whey. Column 4 was treated with wastewater; active microorganisms were present and supplemented with sweet whey. The simulated wastewater solutions described above were sterilized by autoclave and dispensed into the perfusion column medium reservoir. These solutions were continuously recirculated at a rate of approximately 10 - 20 ml/hr. The feed solution was evenly distributed to the soil surface by a pore stone and leached through one kg of soil by gravity. Circulation was driven by premoistened and filtered compressed air.

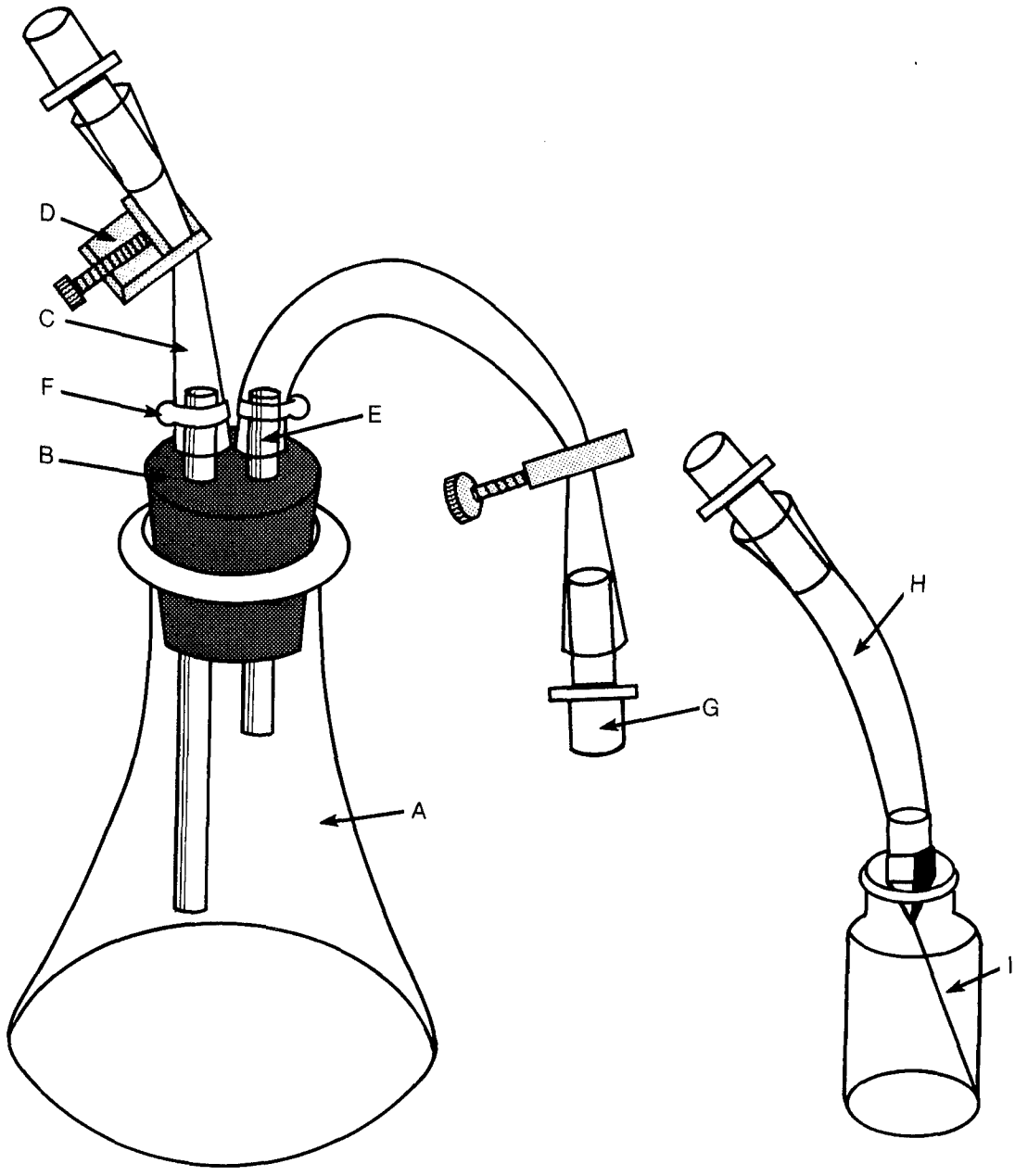
The medium in the reservoir was periodically sampled and analyzed for nitroguanidine. Nitroguanidine was used as an indicator of wastewater biodegradation. After nitroguanidine removal stabilized, the medium was analyzed for all wastewater components and degradation intermediates as described in Subsection 3.2.

3.7 Mineralization.

3.7.1 Apparatus.

3.7.1.1 Nonvolatile compound apparatus. The incubation container was a 125 ml Erlenmeyer flask sealed with a #6 two-hole stopper. Two glass tubes (5 mm O.D.) with attached tygon tubing (2/16 I. D., 5/16 O. D.) and Ty Rap Cable ties (Thomas and Betts) were attached to the stopper. Quick disconnect plugs (VWR) were used to connect the flask with the gassing manifold and collection vial. A syringe needle was attached to one end of the exhaust tube. This needle was placed in a scintillation vial containing trapping solution (Figure 3-3).

3.7.1.2 Volatile compound apparatus. The incubation flasks consisted of 250 ml Erlenmeyer flasks closed with a Teflon-lined screw cap. Two holes were drilled into the screw cap and 16-gauge syringe needles were inserted through the Teflon lining. The lining was cushioned with silicone glue. The syringe needles were secured to the screw cap with epoxy cement. When not in use for flushing the needles were sealed with an epoxy filled syringe barrel. Flasks were confirmed to be airtight by submersion in water for 24 hours. Teflon tubing




A - 125 ml Erlenmeyer flask
B - #6 Two-hole stopper
C - 2/16 I.D., 5/16 O.D. tygon tubing
D - Fixed jaw tubing clamp
E - 5 mm O.D. glass tubing
F - Ty-rap cable tie
G - Quick disconnect plug
H - Exhaust tube with syringe needle
I - Scintillation vial

**Figure 3.3. Non-volatile compound apparatus.**

was used to connect the incubation vessel and the trapping apparatus. The trapping apparatus consisted of scintillation vials held in place by a wooden frame and connected by stainless steel tubing. Scintillation vial caps were cemented to the frame. Each cap was punctured by two stainless steel tubes, one submerged in the fluid and one in the headspaces. Each cap had a Teflon liner cushioned with silicone (Figure 3-4). The system used was described previously by Marinucci and Bartha (16).

3.7.2 Operations.

3.7.2.1 Nonvolatile compound system. Headspace collection from nonvolatile compound flasks was done using positive pressure in a gassing manifold constructed by WESTON to deliver 33 ml/minute of air or nitrogen to 20 experimental flasks. Extraneous $CO_2$ was removed from the displacement gases before entering the incubation flasks. The gases were passed through three scrubber bottles of 10 N NaOH, one indicator bottle of 0.024 N $Ba(OH)_2$, and an empty bottle to trap liquid overflow. $^{14}CO_2$ from flask headspaces was trapped in a solution containing a 1:7 ratio of monoethanol amine and methoxyethanol (Figure 3-5).

3.7.2.2 Volatile compound system. All headspace collection was done under negative pressure using a small aquarium vacuum pump. Aerobic flasks were purged with compressed air every two days for a time period sufficient to allow 7 to 10 flask volume exchanges. Anaerobic flasks were purged with nitrogen for an equivalent time period. The headspace gas was scrubbed in the apparatus described above and shown in Figure 3-6.

Volatile parent and degradation products other than $CO_2$ were trapped in vials $A_1$ and $A_2$ (Figure 3.6) containing Betafluor (National Diagnostics). $^{14}CO_2$ was trapped in vials $O_1$ and $O_2$ containing Oxasol (National Diagnostics). Vials $T_1$ and $T_2$ were kept empty as controls for loss or mixing of trap contents by back pressure. Backflow problems were prevented by connecting the system starting at the source of vacuum (trap $O_2$) and proceeding to the incubation flask. Disconnection proceeded in reverse order, starting at the incubation flask and proceeding to the vacuum source.

3.7.2.3 Swab test for compound adsorption to exposed surfaces. Adsorption of $^{14}C$-labeled guanidine nitrate to exposed surfaces in the test vessel was tested by swabbing the surfaces with filter paper soaked in ethanol. The filter paper was placed in 15 ml of Aquasol-2 scintillation cocktail and counted. Background vials contained clean filter paper to reduce quenching and obtain accurate counts.

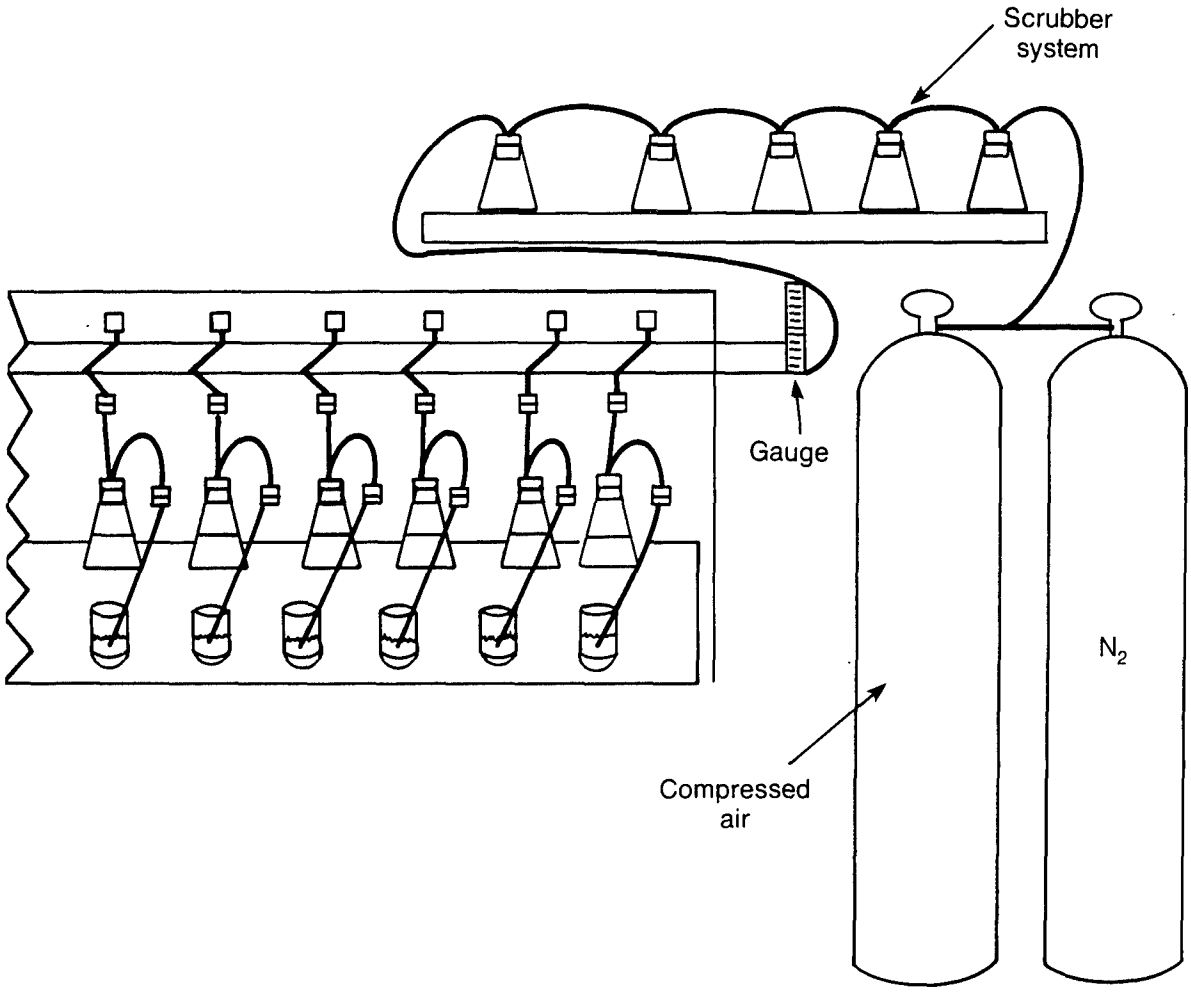
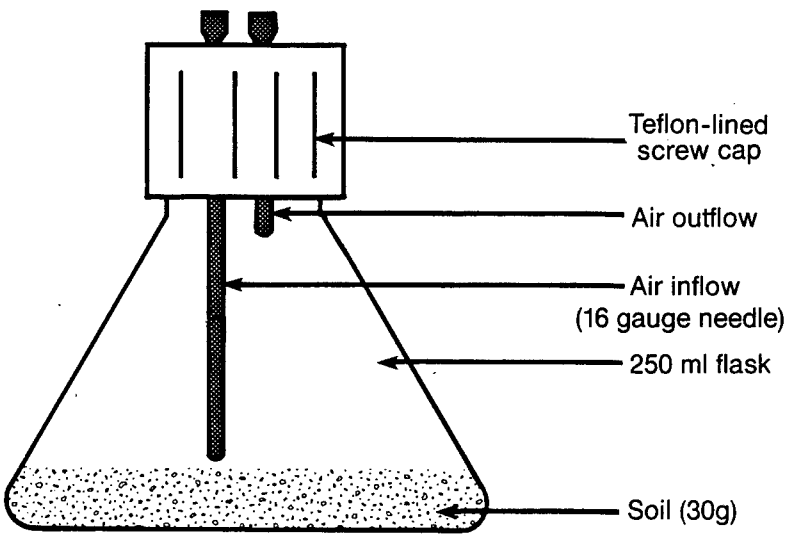
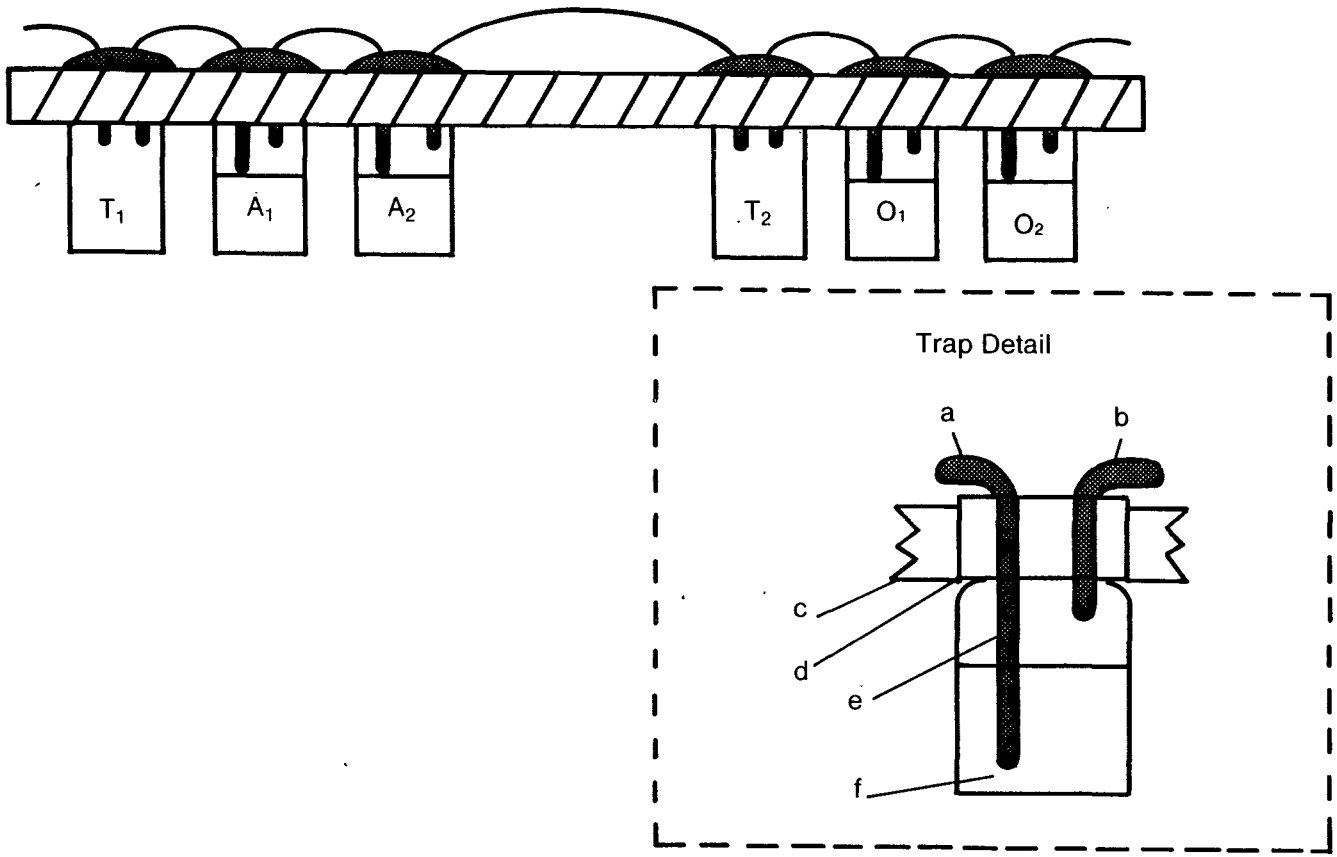



**Figure 3.4. Non-volatile compound system.**




Source: Marinucci and Bartha, 1979

**Figure 3.5. Volatile compound apparatus.**




Source: Marinucci and Bartha, 1979

$T_1$ and $T_2$ are backflow traps. $A_1$ and $A_2$ contain betafluor for guanidine nitrate trapping. $O_1$ and $O_2$ contain Oxasol for $CO_2$ trapping. Trap detail: (a and b) inflow and outflow (1/16 in. O.D.); (c) wood mounting board; (d) scintillation vial caps; (e) glass scintillation vial; (f) trapping fluor.

**Figure 3.6. Volatile compound system.**

3.7.2.4 Alkali test for volatile compounds. Evolved $^{14}C$ was determined to be $^{14}CO_2$ by the following procedure. $^{14}C$ was trapped in 15 ml of alkali (1 N NaOH) and mixed with 5.0 ml of barium chloride (3.8 g/l $BaCl_2$). The precipitated $CO_2$ ($BaCO_3$) was collected by filtration. The sample was counted after the scintillation cocktail was added and chemiluminescence eliminated by 24 hours incubation in the dark at 4°C.

3.7.3 Experimental procedures. Mineralization of the $^{14}C$-labeled substrate to $^{14}CO_2$ was followed in accordance with Standard OECD (17) and EPA (18) guidelines. Three replicates were run for each test. Additives were dissolved in the smallest volume of water possible. Typical additives to the flask mineralization studies included 1.0 ml of nutrient solution, 1.0 ml of microbial inoculum, 0.1 - 0.2 ml of $^{14}C$-labeled substrate, 1.0 ml of 1.0 percent simulated wastewater, 1.0 ml of a 0.1 percent carbon supplement solution, and 1.5 ml of deionized water. All additives were presterilized. $^{14}C$-labeled substrate additions were made to achieve the following $^{14}C$ values per flask: amino acid, $1.1 \times 10^5$ dpm; glucose, $1.0 \times 10^5$ dpm; guanidine nitrate, $4.0 \times 10^5$ dpm; and nitroguanidine, $4.0 \times 10^5$ dpm. Soil was added to the flask after appropriate quantities of test substrate and additives so that the soil was moistened from below by capillary action to obtain approximately 60 percent water holding capacity. Mineralization flasks were sealed and incubated at 20°C for aerobic tests. Anaerobic tests were incubated at room temperature in a Coy (Coy Manufacturing) anaerobic chamber. Purges were done with a frequency that maintained oxygen within aerobic mineralization rate test systems. Anaerobic tests were sparged with nitrogen and all handling operations conducted in a Coy anaerobic chamber using deaerated solutions.

To ensure anaerobiosis, all anaerobic mineralization flasks were established in the anaerobic chamber. All equipment was allowed to equilibrate within the anaerobic chamber for two hours before use. Anaerobic conditions within the chamber were monitored with anaerobic indicator media.

In order to maintain a suitable ratio of isotopic to non-isotopic test material, a 0.1 percent wastestream chemical background was utilized. The base (wastewater background) concentrations for NQ and GN in test soils were 2.5 ppm and 0.20 ppm, respectively. When isotopic NQ was added, the final NQ concentration was 2.57 ppm, and when isotopic GN was added, the final GN concentration was 0.28 ppm. The experimental ratio of nonisotopic NQ to isotopic NQ was 37:1, and the ratio of nonisotopic GN to isotopic GN was 2.5:1.

The reduction in wastestream composition required an adjustment in carbon supplementation. One g/l of glucose or whey was added to a 5 percent simulated wastestream for the column studies. In order to maintain this ratio, a 20 mg/l supplement level was used for the 0.1 percent simulated wastestream. Additives were adjusted to neutrality before use in mineralization studies.

Rate of mineralization controls were heat sterilized on three consecutive days to ensure uniform inactivity of microorganisms and mercuric chloride-inactivated to prevent contamination during headspace purges of the soil flasks.

3.8 Enumeration.

3.8.1 Plate counts. Enumerations were performed by tenfold serial dilution and standard plate count methods (12). Plates were incubated at 35°C for 48 hours before counting. All manipulations were performed in a laminar flow hood.

3.8.2 $^{14}$C-MPN. All $^{14}$C-MPN enumerations were based on 5 replicates of media inoculated with successive 10-fold sample dilutions and incubated for 6 weeks at 24°C (19). The growth medium was autoclaved soil extract water with 5-25 µg/l of $^{14}$C-labeled substrate. Five replicates, containing 1 ml of medium in an cotton-plugged biovial (Beckman, Inc.) placed inside a standard glass scintillation vial, were prepared and sterilized for each sample dilution to be enumerated. These tubes were inoculated with a 0.1 ml sample of the appropriate serial dilution, the sterile cotton plugs replaced, and the biovial placed in a scintillation vial containing 1 ml of 1 N NaOH for trapping evolved $^{14}CO_2$. Sterile controls were used to correct for background radioactivity.

After 6 weeks of incubation, biovials were removed and discarded. Ten milliliters of PCS® scintillation cocktail (Amersham) were added to each scintillation vial and chemiluminescence was eliminated by 48 hours of incubation in the dark at 4°C. Samples were gelled by adding 4 ml of water and then counted. Replicates evolving at least 1 percent of the added dpm (after subtraction of disintegrations per minutes for controls) were scored positive.

3.9 Soil mobility study.

3.9.1 Apparatus. A plexiglass column (6.5 cm I. D. x 10 cm L) with a fine stainless steel screen (50 mesh) and a coarse stainless steel screen (20 mesh) at the top and bottom to hold soil in place was enclosed with screw-tightened plexiglass lids and O-ring seals. The column included an influent and effluent port at bottom and top as well as a flushing port at the bottom. The apparatus is shown in Figure 3-7.

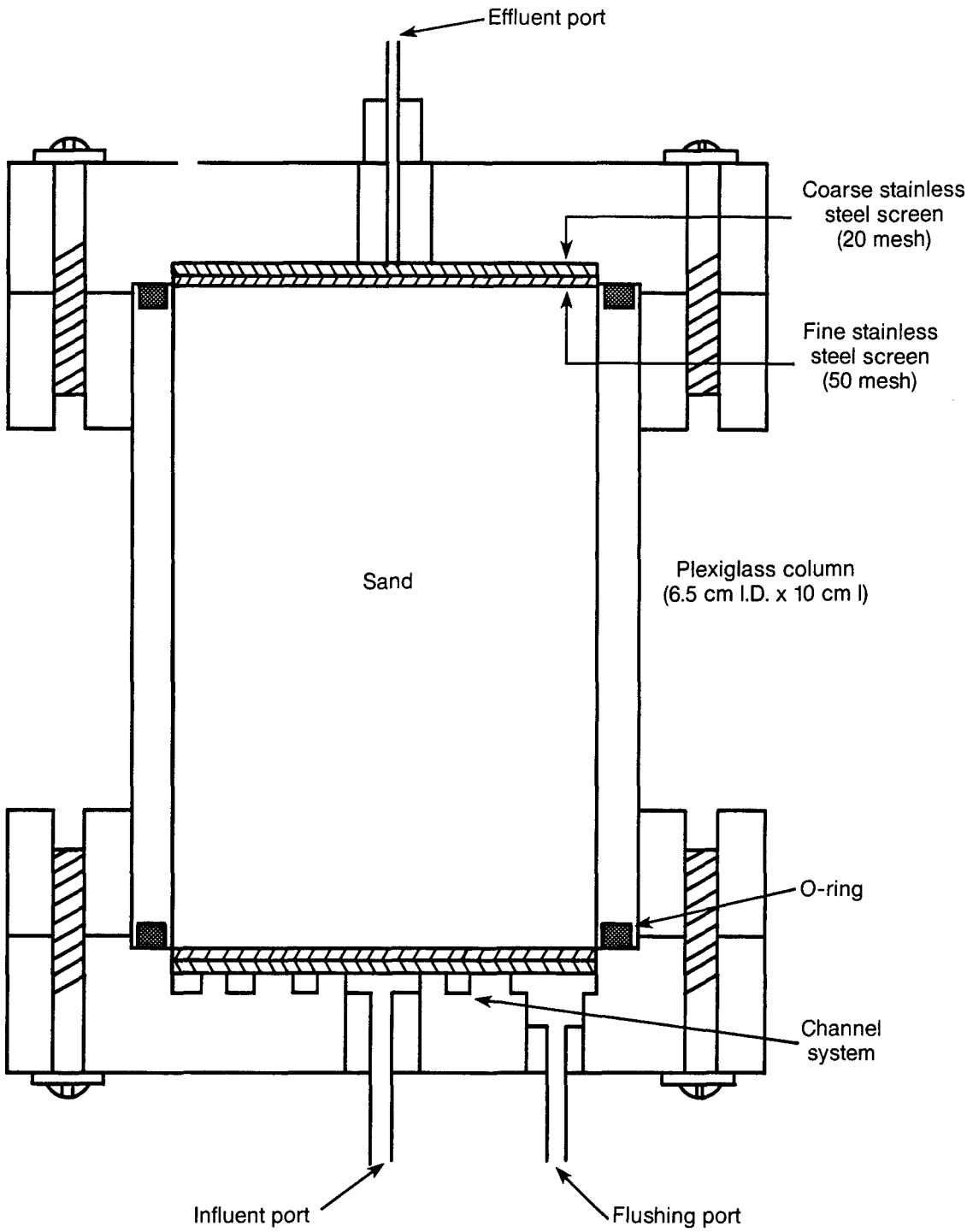



**Figure 3.7. Soil mobility column.**

3.9.2 Operation. Flow to the columns was controlled by a peristaltic pump. A manometer was included in the influent line to monitor any changes in the influent pressure over the course of the study. Effluent samples were continuously collected in scintillation vials (Figure 3-8). Several pore volumes of solution not containing test materials were passed through the columns prior to using test materials. Flow rates of 4 ml/hr were used. A chloride tracer was used prior to introduction of the test materials to characterize the column performance. A 0.1 pore volume pulse (~16 ml) of sodium chloride solution (1650 mg/l) was added to the column inlet. Chloride in the effluent was measured using an Orion Chloride Electrode. The quantity of the chloride found in the effluent was within ±25 percent of the dosed quantity.

After dosing with $^{14}C$-labeled test material (approximately 500,000 dpm per column), effluent samples were quantified for $^{14}C$ content and volume. Once effluent samples returned to near background levels, soil combustions were performed on samples from top, middle, and bottom sections of the soil columns. $^{14}C$-mass balance calculations were performed. All influent solutions contained 0.75 percent mercuric chloride to ensure sterility. Sterility was verified by plate count and inoculation of growth media.

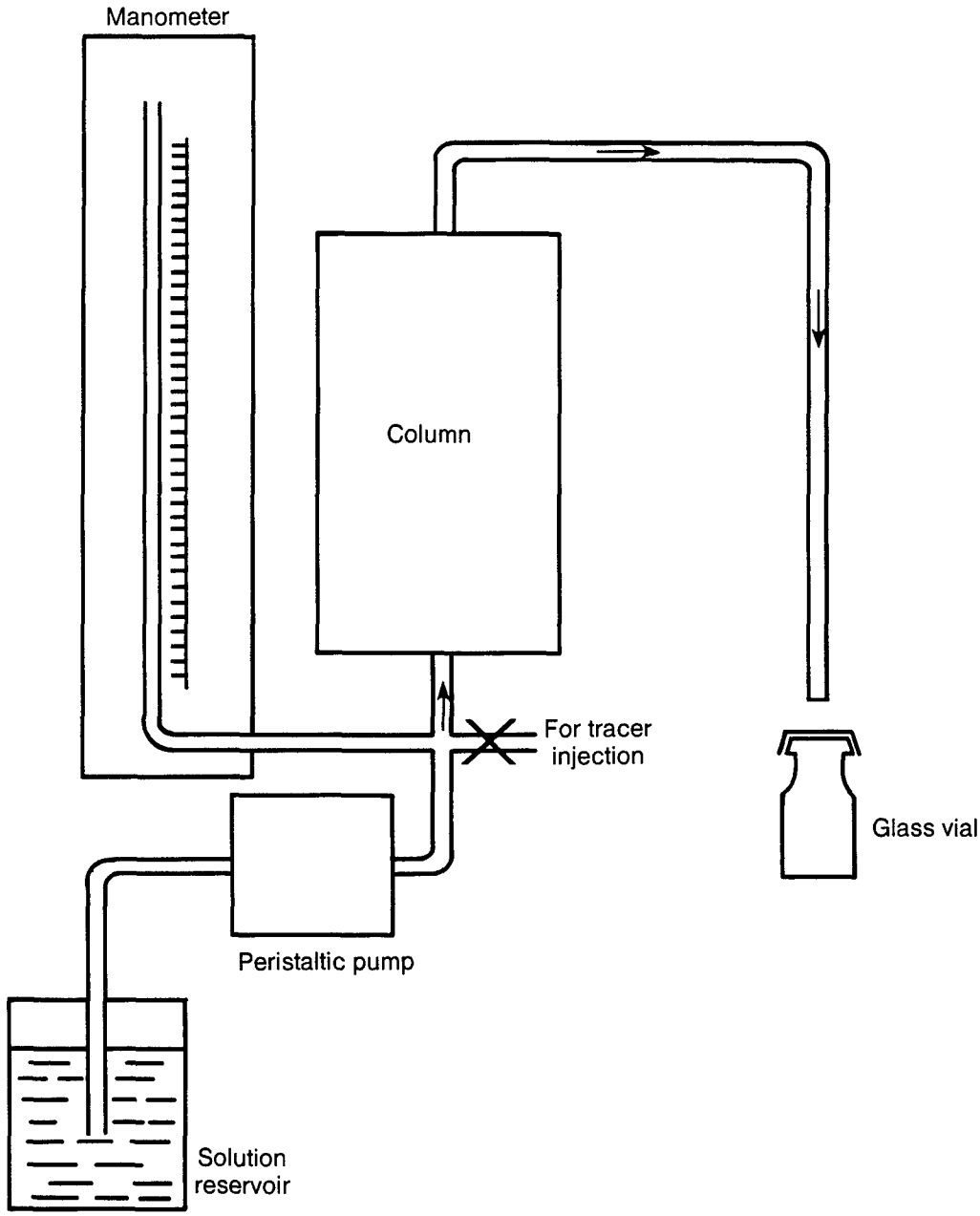



**Figure 3.8. Soil mobility column and collection system.**

## 4. RESULTS

### 4.1 Continuous flow soil columns.

4.1.1 Experimental design. The fate of NQ wastewater components in SFAAP soil was monitored in continuous flow soil columns. This system was based upon a system used by Kaplan and Kaplan (7), and was designed to simulate land application system. Six columns were established with the following parameters:

| No. | Medium | Microorganisms | Supplement |
|---|---|---|---|
| 1 | Tap water | Active | None |
| 2 | Wastewater | Active | None |
| 3 | Wastewater | Sterile | Glucose |
| 4 | Wastewater | Active | Glucose |
| 5 | Wastewater | Sterile | Whey |
| 6 | Wastewater | Active | Whey |

The logic for these individual column parameters was as follows:

No. 1 - This column is an experimental control that mimics natural leaching in SFAAP soil expected as a result of rainfall.
No. 2 - This column determines the amount of NQ wastewater degradative activity that occurs without carbon supplementation. Activity observed is the sum total of biological and nonbiological degradation.
No. 3 - This column serves as a control for column number 4.
No. 4 - This column reveals the total amount of degradation facilitated by glucose when compared with column 2 and the amount of biological degradation facilitated by glucose when compared with column 3.
No. 5 - This column serves as a control for column number 6.
No. 6 - This column reveals the total amount of degradation facilitated by whey when compared with column 2 and the amount of biological degradation facilitated by whey when compared with column 5.

The columns were operated for a period of 271 days. Influents were analyzed every 3 weeks for the first 118 days, and every other week for the remainder of the test period. Effluents were analyzed every week for the first 118 days, and every other week for the remainder of the test period.

At the end of the test period the columns were dismantled and the soil was analyzed for a variety of chemical and biological parameters, including the occurrence of microbial adaptation.

Changes observed in wastewater constituents and intermediates are graphically presented and statistically analyzed. Only illustrative data are presented in the text. Figures 4-1 through 4-4 illustrate typical influent and effluent wastewater component levels in the continuous flow soil columns. Appendix B contains graphs of influent and effluent data for all columns. Appendix C is a tabular presentation of all column data collected. The graphs and data table show a drop in NQ levels for day 90 influent. The drop is probably due to omission of NQ in that batch of simulated wastewater. Column 1 on day 38 inadvertently received wastewater components.

4.1.2 Nitroguanidine. A decrease in nitroguanidine levels from influent to effluent was observed within columns 4, 5, and 6. Based on mean values, there was a 30 to 40 percent reduction (Table 4-1) from influent to effluent in these columns. Statistical comparisons of wastewater influent and effluent for individual components within each column were made using univariate analysis. Normal distribution was assumed for each component. The Z values were calculated to determine significant differences between influent and effluent levels for each column (Appendix D). The Z values for nitroguanidine in columns 4, 5, and 6 demonstrate a significant difference between influent and effluent NQ for each of these columns. Z values of greater than 1.96 or less than -1.96 indicate significant differences with 95 percent confidence level (Table 4-2).

Comparison of effluent concentrations between columns was conducted using regression analysis. It was assumed that effluent content was a function of influent content and time. Analysis was conducted to determine whether the different soil column treatments had a significant impact on the assumed functional relationship between influent and effluent (Appendices E, F, and G). The homogeneity of two regressions was tested to determine whether the parameters of linear regression used to represent the functional relationship between influent and effluent remained stable under two different column treatment conditions (20). F-test values were calculated using the null hypothesis that a wastewater component follows the same regression parameters in both columns (Appendix G).

When NQ changes in columns 4, 5, and 6 were compared to changes in column 2 (wastewater without carbon supplement), no significant differences were observed. This comparison was run using an F-test. Significant differences are indicated by F-test values listed in Table 4-3 with alpha-type error at 5 percent level. Using the same F-test, comparison of active and

TABLE 4-1. PERCENT CHANGE OF WASTEWATER COMPONENTS IN SFAAP SOIL

| Component | Soil column 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| NQ | - 43.27 | -1.26 | -6.90 | -30.90 | -29.00 | -44.32 |
| NOQ | 0 | +340.00 | +1200.00 | +282.00 | +1335.00 | +1478.00 |
| CY | + 3.44 | -12.80 | -29.70 | -21.90 | -34.70 | -35.60 |
| G | -100.00 | -90.00 | -91.00 | -61.00 | -100.00 | -86.00 |
| $NO_2$-$NO_3$ | +157.14 | +5.70 | -25.30 | -31.16 | -14.40 | -24.80 |
| $NH_3N$ | + 92.00 | -97.00 | -0.52 | +61.00 | -53.00 | +27.00 |
| $SO_4$ | --- | -2.30 | -14.57 | -68.26 | -4.20 | -49.80 |
| TOC | +233.18 | +22.80 | +32.20 | -52.40 | +8.80 | -69.50 |

Key:

NQ - Nitroguanidine
NOQ - Nitrosoguanidine
CY - Cyanamide
G - Guanidine
$NO_2$-$NO_3$ - Nitrite-nitrate
$NH_3$-N - Ammonia-nitrogen
$SO_4$ - Sulfate
TOC - Total organic carbon

TABLE 4-2. Z-TEST VALUES CONTINUOUS FLOW SOIL COLUMNS

| Component | Soil columns 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| Nitroguanidine | -0.37 | 0.17 | 2.42 | -2.34 | -3.95 |
| Nitrosoguanidine | -1.48 | -1.82 | -1.34 | -1.76 | -2.50 |
| Cyanamide | 0.95 | 0.32 | 1.63 | 0.96 | 2.35 |
| Guanidine | 2.05 | 3.35 | 1.40 | 3.66 | 2.18 |
| Nitrite-Nitrate | 0.16 | 0.76 | 1.89 | 0.82 | 1.13 |
| Ammonia nitrogen | 7.18 | 0.46 | -1.48 | 1.85 | -1.30 |
| Sulfate | -0.25 | 0.81 | 6.76 | -0.074 | 3.49 |
| Total Organic Carbon | -3.34 | 1.13 | 2.83 | -0.53 | 5.51 |

Significant = ±1.96
See Appendix D, Calculation of Z Value

TABLE 4-3. F-TEST VALUES CONTINUOUS FLOW SOIL COLUMNS

| Component | Soil columns | | | | | |
|---|---|---|---|---|---|---|
| | 2-3 | 2-4 | 2-5 | 2-6 | 3-4 | 5-6 |
| Nitroguanidine | 1.89 | 2.00 | 2.16 | 2.40 | 0.20 | 0.35 |
| Cyanamide | 2.78 | 0.82 | 5.23 | 1.62 | 1.72 | 1.31 |
| Nitrite-Nitrate | 1.74 | 3.12 | 0.86 | 0.29 | 3.99 | 1.50 |
| Ammonia nitrogen | 27.12 | 0.77 | 14.97 | 2.43 | 2.02 | 1.17 |
| Total Organic Carbon | 15.75 | 3.03 | 1.74 | 6.71 | 0.92 | 7.39 |

F 0.05, = 3.16
See Appendix G, Statistical Procedure for Testing Homogeneity 0 and Two Regressions

Continuous flow soil column influent explosives

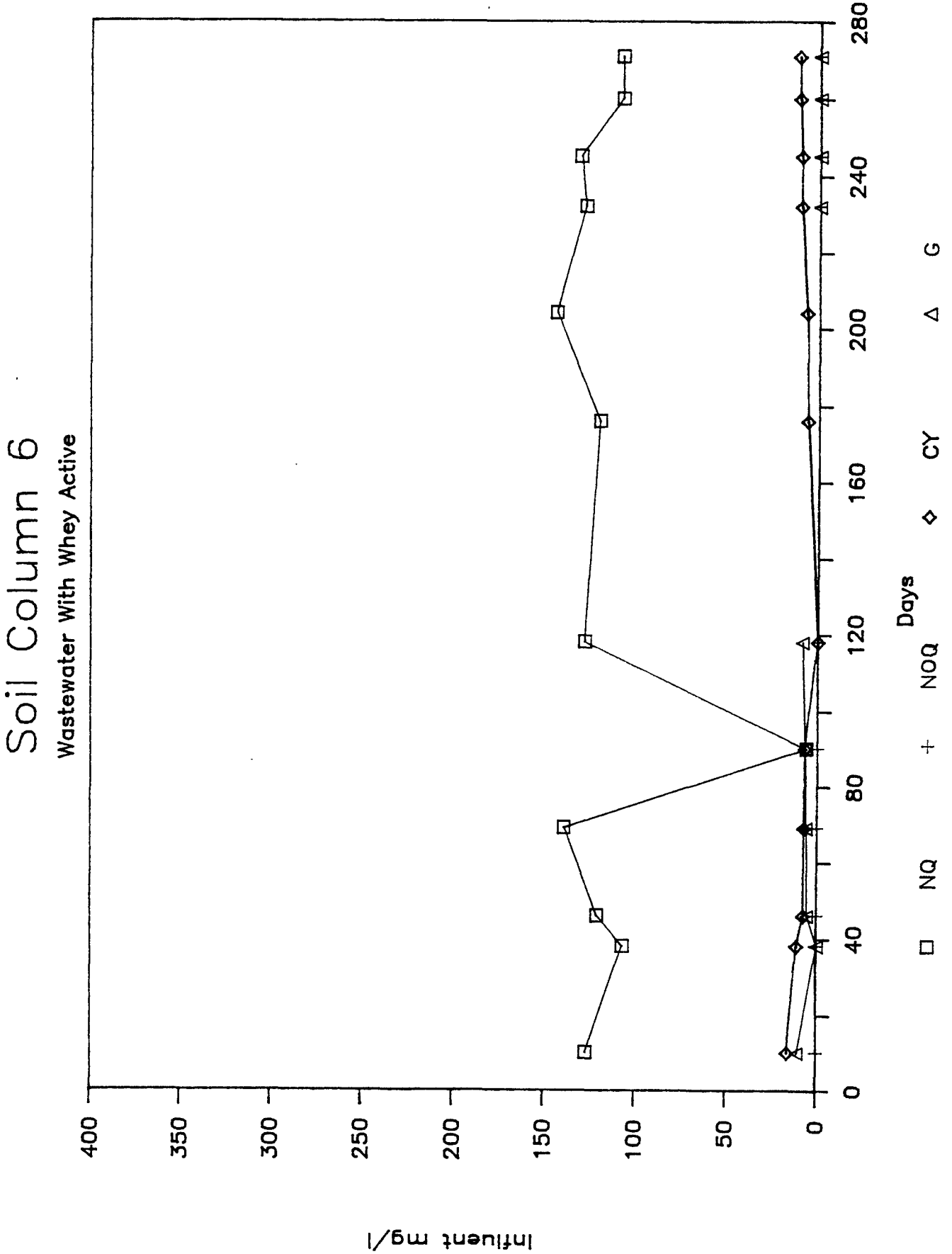
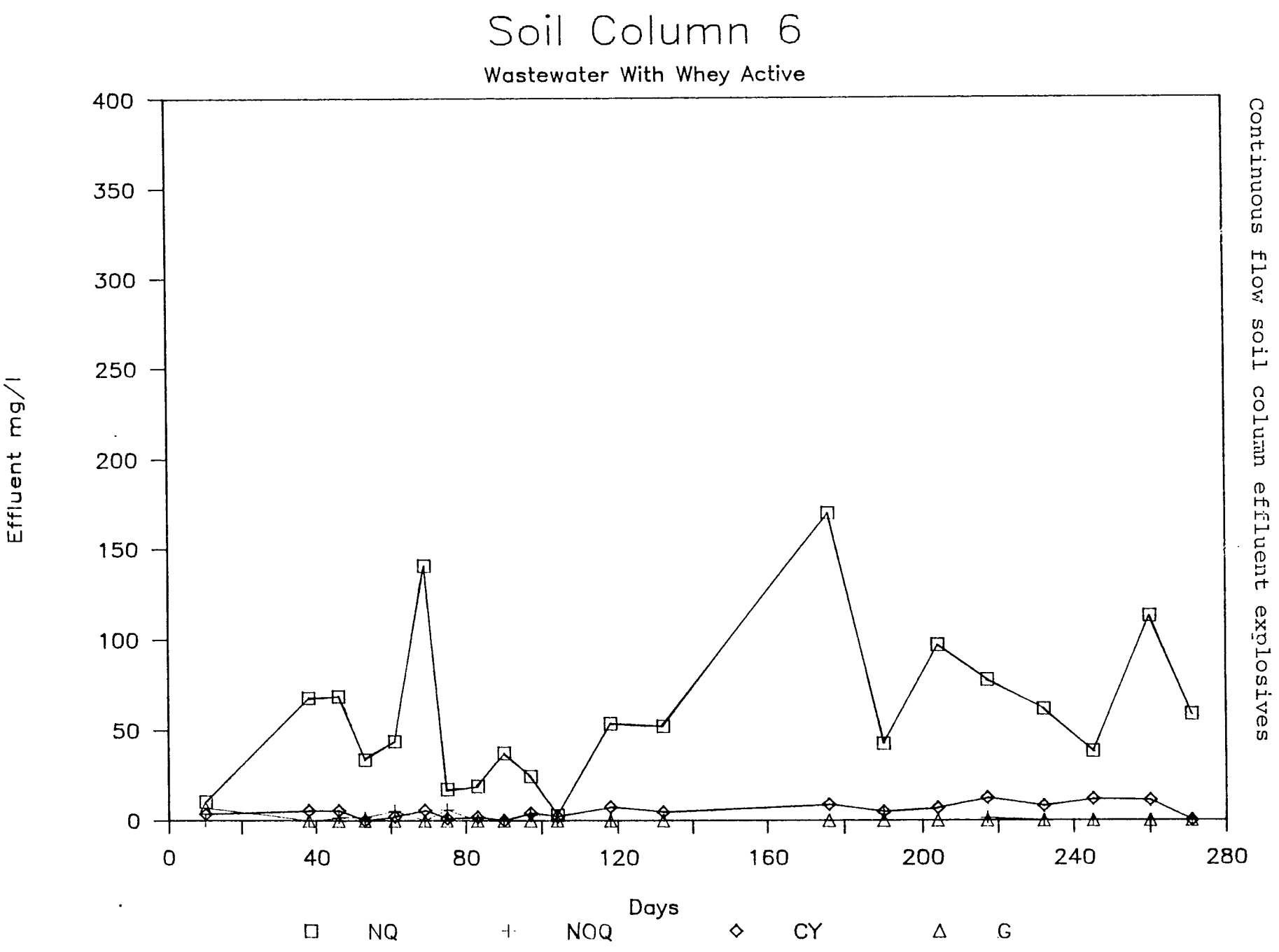



Figure 4-1

Figure 4-2


Soil Column 6
Wastewater With Whey Active
Effluent mg/l
Continuous flow soil column effluent explosives
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

Continuous flow soil column influent non-explosives

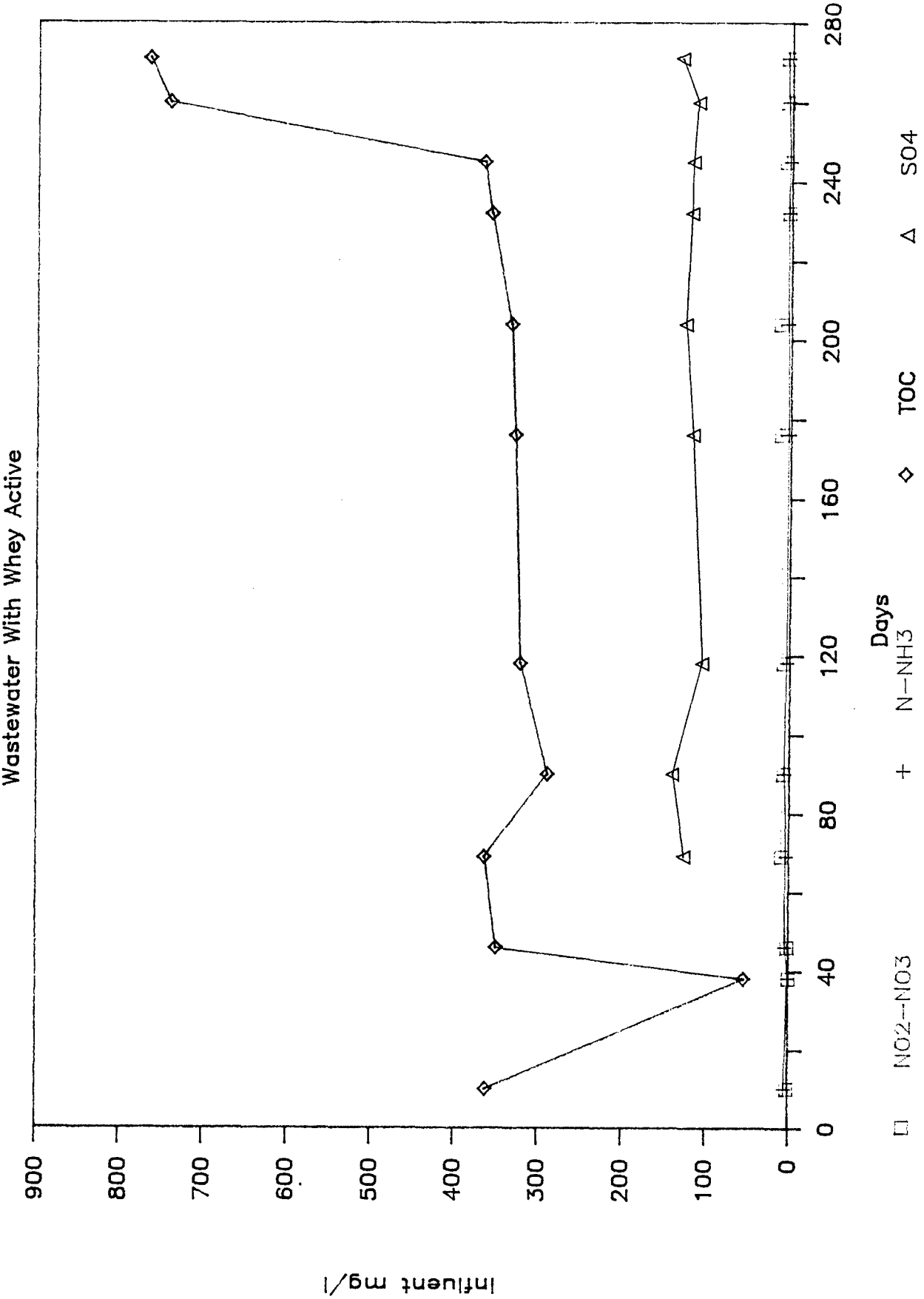
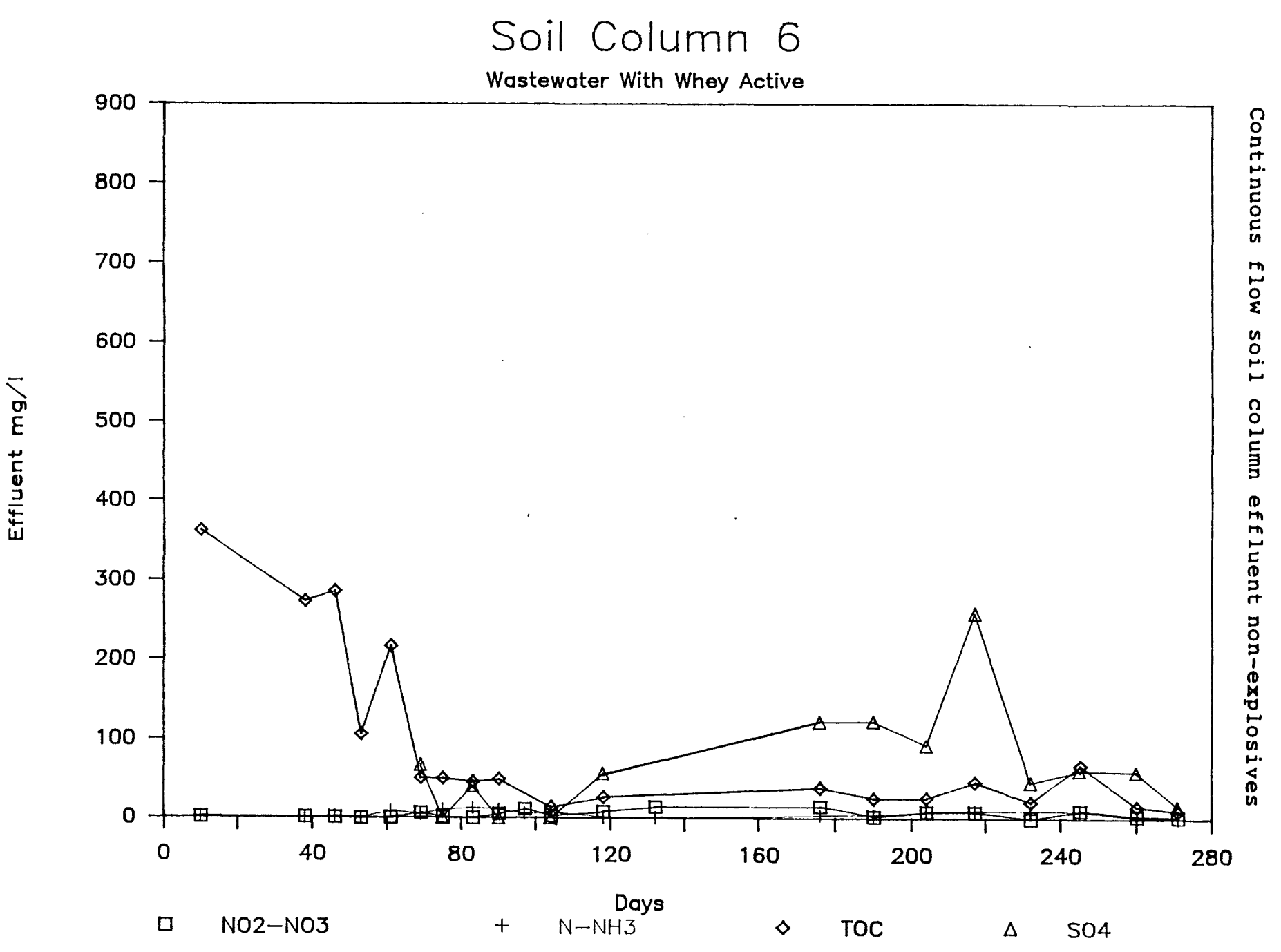



Figure 4-3

Figure 4-4


Soil Column 6
Wastewater With Whey Active
Effluent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
Continuous flow soil column effluent non-explosives
NO2—NO3
N—NH3
TOC
SO4

sterile columns (3 with 4 and 5 with 6) did not indicate significant transformation of NQ.

4.1.3 Nitrosoguanidine. The level of nitrosoguanidine increased from influent to effluent within all columns, (Table 4-1), except column one which remained below detection limits of 0.2 mg/l. Increases ranged from 282 to 1,478 percent based on mean values. The mean NOQ influent level in columns with wastewater addition was 0.04 mg/l. The mean NOQ effluent level in columns with wastewater addition was 0.34 mg/l. NOQ data are not readily visible on graphs in Appendix B, due to graph scale, but data are presented in tabular form in Appendix C.

4.1.4 Cyanamide. The level of cyanamide decreased from influent to effluent within columns 2 through 6 (Table 4-1). The only statistically significant reduction, however, occurred in column 6 (active with whey supplement), as indicated by Z values (Table 4-2). When columns were compared with each other, using an F-test, a significant difference was found between the effluents of columns 2 and 5. Comparison of other columns indicated no significant differences in effluent cyanamide content (Table 4-3). Cyanamide levels in column 1 remained near or below detection limits (2.0 mg/l) throughout the study.

4.1.5 Guanidine. The level of guanidine decreased between 61 to 100 percent from influent to effluent within all columns except column 1 (Table 4-1). This decrease was significant in columns 2, 3, 5 and 6 as determined by Z value (Table 4-2). No F-test comparisons between column effluents were conducted for guanidine.

4.1.6 Nitrite-nitrate. Based on mean values, nitrite-nitrate levels decreased between 14 to 31 percent from influent to effluent within columns 3, 4, 5 and 6. Column 1 and 2 had increases in nitrite-nitrate from influent to effluent (Table 4-1). However, no statistically significant changes occurred from influent to effluent when each column was separately analyzed (Table 4-2).

When column effluents were compared to each other by an F-test, differences were found in nitrite-nitrate levels for column 2 compared to column 4 and column 3 compared to column 4 (Table 4-3), which indicate the column soils had a significant impact on the assumed functional relationship between influent and effluent.

4.1.7 Ammonia. Varied results were observed in carbon supplemented columns. Ammonia increased from influent to effluent within active columns (numbers 4 and 6) and decreased from influent to effluent in sterile columns (numbers 3 and 5) based on mean values (Table 4-1). However, none of these differences was significant based on Z value (Table 4-2).

Ammonia did decrease significantly from influent to effluent in column 2 (active unsupplemented wastewater), as indicated by Z value in Table 4-2. When the influent and effluent ammonia levels in column 2 were compared to columns 3 and 5, significantly more ammonia was present in the effluent of columns 3 and 5, based on F-test values. No other significant differences in ammonia levels were found from comparisons between columns (Table 4-3). Ammonia levels in column 1 remained near or below the detection limit (0.03 mg/l) throughout the study.

4.1.8 Sulfate. Significant transformation of sulfate occurred in the microbially active columns, but not in the sterile columns (Table 4-2). Carbon supplementation facilitated sulfate removal, with a 68 percent reduction observed in column 4 and a 50 percent reduction in column 6. Column 2 (active, no supplement) had only a 2 percent sulfate reduction (Table 4-1). Strong odor was emitted from column 6 but no black sediment (FeS) ferrous sulfide was observed. Column 4 did not have a strong odor or black sediment. The inoculum column, which had a smaller volume and tendency to become waterlogged, had a characteristic hydrogen sulfide odor and black sediment.

4.1.9 Total organic carbon. Reductions of greater than 50 percent total organic carbon were observed from influent to effluent in active columns 4 and 6 based on mean values (Table 4-1). These differences were significant, as illustrated by Z values reported in Table 4-2.

Comparison of columns to each other by F-test revealed significant differences in TOC when comparing column 2 with column 3, 2 with 6, and 5 with 6, but not when comparing 2 with 5, or 3 with 4. Comparison of column 2 with 4 approached a significant difference (Table 4-2). TOC levels in column number 1 remained low. Mean influent TOC for column number 1 was 2.14 mg/l. Mean effluent TOC for column number 1 was 7.13 mg/l. A dip in TOC influent level on day 46 is the result of cumulative sterility problems during the initial period of the test in reservoirs containing glucose or whey. Microbial activity in the influent reservoirs caused TOC reductions.

Increases in TOC levels from day 240 until the end of the study are due to a doubling of carbon supplement in simulated wastewater reservoirs in an effort to enhance cometabolism. No change in transformation of wastewater components was observed after these increases in carbon supplement.

4.1.10 Cyanoguanidine and melamine. These two intermediates of NQ transformation were not detected in influent or effluent samples from any of the columns during the course of the study. Detection limits for the two compounds were 0.2 mg/l and 2.0 mg/l, respectively.

4.1.11 Total nitrogen balance. Total nitrogen in the continuous flow soil column systems was calculated from wastewater components, NQ and GN intermediates and total nitrogen in the soil. Soil extract analysis is reported in Table 4-4. The nitrogen forms accounted for in these calculations include ammonia, nitrite-nitrate, nitroguanidine, guanidine, nitrosoguanidine, cyanamide, and total nitrogen. The percentage of added plus background soil nitrogen accounted for by effluent plus post treatment soil nitrogen was 99 percent for column 1; 84 percent, column 2; 107 percent, column 3; 109 percent, column 4; 96 percent, column 5; and 87 percent, column 6. See Appendix H for a complete presentation of nitrogen mass balance calculations.

4.1.12 pH. The SFAAP soil, as received, had a pH of 7.1. The pH values of the column soils after treatment were: column 1, 6.8; column 2, 6.5; column 3, 4.9; column 4, 5.5; column 5, 5.7; column 6, 6.7 (Table 4-5).

Influent and effluent pH values were monitored over the course of the study (Table 4-6). Column 1 (deionized water) had influent pH between 5.0 - 7.0, and effluent pH between 8.0 - 8.2. Columns 2, 3, 4, 5, and 6 (wastewater treatment) had influent pH between 3.5 and 6.8 and effluent pH between 4.8 - 8.3. Columns 3 and 5, containing wastewater sterilized with mercuric chloride, had the lowest pH for influent samples (3.5 - 5.6) and effluent samples (4.8 - 6.4). No other significant trends, changes, or fluctuations were observed in the pH of the soil influent or effluent.

4.1.13 Viability of soil column influent and effluent. The presence of microorganisms in the soil column influents and effluents was monitored throughout the study (Table 4-7). Soil column influent reservoirs remained sterile throughout the experiment with the exception of column 4 on day 90, and column 6 on days 53, 69, 90, 97, 104, 217, and 231. When contamination was detected, the reservoir was immediately replaced with the appropriate sterile solution.

TABLE 4-4. SOIL EXTRACTION
(mg/kg)

| Components | Pretreatment Soil | Post-Treatment Soil Column 1 Top | 1 Bottom | 2 Top | 2 Bottom | 3 Top | 3 Bottom | 4 Top | 4 Bottom | 5 Top | 5 Bottom | 6 Top | 6 Bottom |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NQ | 0 | 0 | 0 | 55 | 58 | 73 | 54 | 11 | 0 | 42 | 31 | 0 | 0 |
| NOQ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | --- | 290 | 220 | 693 | 430 | 563 | 275 | 1,600 | 235 | 330 | 380 | 1,710 | 180 |
| GN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CYG | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOC | 8,830 | 8,810 | 8,850 | 14,000 | 4,300 | 7,770 | 13,800 | 7,900 | 9,320 | 13,200 | 5,640 | 5,180 | 6,770 |
| TKN | 753 | 605 | 901 | 813 | 1,230 | 973 | 1,750 | 1,670 | 1,610 | 1,600 | 1,610 | 931 | 1,640 |

TABLE 4-5. CONTINUOUS FLOW SOIL COLUMNS - SOIL pH

| Sample description | | pH |
|---|---|---|
| SFAAP soil pretreatment | | 7.1 |
| SFAAP soil post treatment | Column 1 | 6.8 |
| | Column 2 | 6.5 |
| | Column 3 | 4.9 |
| | Column 4 | 5.5 |
| | Column 5 | 5.7 |
| | Column 6 | 6.7 |

TABLE 4-6. CONTINUOUS FLOW SOIL COLUMNS pH MONITORING

| Column | Day 10 | 46 | 69 | 100 | 133 | 203 | 221 | 232 | 242 | 252 | 259 | 265 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Influent | 5.1 | 5.0 | 7.0 | 6.0 | 6.1 | 6.1 | 6.6 | 5.0 | 6.8 | 7.0 | 7.0 | 6.0 | 7.0 |
| 1 Effluent | 8.0 | 8.1 | 8.2 | 8.0 | 8.0 | 8.0 | 8.2 | 8.0 | 8.1 | 8.2 | 8.0 | 8.0 | 8.0 |
| 2 Influent | 6.0 | 6.0 | 6.4 | 6.7 | 6.0 | 6.0 | 6.0 | 6.8 | 6.0 | 6.0 | 6.4 | 6.7 | 5.5 |
| 2 Effluent | 8.0 | 8.0 | 8.1 | 8.2 | 8.3 | 8.0 | 8.0 | 8.0 | 8.0 | 8.1 | 8.0 | 8.0 | 8.0 |
| 3 Influent | 3.5 | 4.0 | 4.0 | 4.0 | 4.0 | 3.5 | 3.5 | 4.5 | 4.0 | 4.2 | 4.0 | 4.0 | 4.0 |
| 3 Effluent | 5.0 | 5.0 | 5.0 | 5.1 | 5.5 | 5.0 | 4.8 | 5.0 | 5.0 | 5.1 | 5.0 | 5.0 | 5.0 |
| 4 Influent | 6.2 | 5.7 | 6.6 | 5.9 | 5.5 | 6.2 | 6.8 | 5.7 | 6.2 | 6.6 | 5.5 | 4.9 | 6.2 |
| 4 Effluent | 6.5 | 6.5 | 6.6 | 6.5 | 7.7 | 6.5 | 6.6 | 6.7 | 6.5 | 6.6 | 6.5 | 6.5 | 6.5 |
| 5 Influent | 4.0 | 4.5 | 4.5 | 4.7 | 4.4 | 4.0 | 4.2 | 4.5 | 4.5 | 4.7 | 4.9 | 5.6 | 4.7 |
| 5 Effluent | 6.0 | 6.1 | 6.3 | 6.1 | 6.4 | 6.0 | 6.0 | 6.0 | 6.1 | 6.1 | 6.0 | 6.0 | 6.0 |
| 6 Influent | 6.0 | 6.0 | 6.3 | 6.4 | 6.5 | 6.0 | 6.4 | 6.3 | 6.5 | 6.4 | 6.4 | 6.3 | 6.5 |
| 6 Effluent | 8.0 | 8.0 | 8.0 | 8.0 | 8.2 | 8.0 | 8.0 | 8.0 | 8.1 | 8.2 | 7.8 | 8.0 | 8.0 |

TABLE 4-7. CONTINUOUS FLOW SOIL COLUMN - PLATE COUNTS

| Column | Day 53 Date 1/28 | 61 2/7 | 69 2/14 | 83 2/27 | 90 3/6 | 97 3/13 | 104 3/21 | 132 4/18 |
|---|---|---|---|---|---|---|---|---|
| 1 - Influent | 0 | ~* | 0 | ~ | 0 | 0 | 0 | 0 |
| 2 - Influent | 0 | ~ | +* | ~ | 0 | 0 | 0 | 0 |
| 3 - Influent | 0 | ~ | 0 | ~ | 0 | 0 | 0 | 0 |
| 4 - Influent | 0 | ~ | 0 | ~ | $6.0 \times 10^4$ | 0 | ~ | 0 |
| 5 - Influent | 0 | ~ | 0 | ~ | 0 | 0 | 0 | 0 |
| 6 - Influent | + | ~ | + | ~ | $3.1 \times 10^6$ | $2.75 \times 10^6$ | $1.1 \times 10^4$ | 0 |
| 1 - Effluent | + | $3.2 \times 10^3$ | $3.5 \times 10^3$ | ~ | $6.5 \times 10^3$ | ~ | ~ | $4.7 \times 10^5$ |
| 2 - Effluent | + | $8.6 \times 10^2$ | $3.0 \times 10^3$ | $4.9 \times 10^3$ | $1.5 \times 10^4$ | ~ | ~ | $1.6 \times 10^4$ |
| 3 - Effluent | + | $4.3 \times 10^5$ | $1.0 \times 10^2$ | 0 | 0 | ~ | ~ | 0 |
| 4 - Effluent | + | $3.3 \times 10^5$ | $7.8 \times 10^5$ | $4.8 \times 10^6$ | 0 (3+4 samples mixed) | ~ | ~ | $1.9 \times 10^5$ |
| 5 - Effluent | + | $3.44 \times 10^6$ | $1.0 \times 10^2$ | 0 | 0 | ~ | ~ | 0 |
| 6 - Effluent | + | $2.46 \times 10^6$ | $1.6 \times 10^7$ | $9.6 \times 10^6$ | $4.6 \times 10^6$ | ~ | ~ | $3.8 \times 10^6$ |

*~ - Not plated
*+ - Growth present but not enumerated.

TABLE 4-7. (CONTINUED)

| Column | Day / Date | 190 5/15 | 204 5/29 | 217 1/28 | 231 2/7 | 245 2/14 | 249 2/27 | 260 3/6 |
|---|---|---|---|---|---|---|---|---|
| 1 - Influent | | ~ | 0 | 0 | 0 | ~ | ~ | 1 |
| 2 - Influent | | ~ | 0 | 0 | 0 | ~ | ~ | 1 |
| 3 - Influent | | ~ | 0 | 0 | 0 | ~ | ~ | 0 |
| 4 - Influent | | ~ | 0 | 1 | 0 | 0 | 0 | 0 |
| 5 - Influent | | ~ | 0 | 0 | 0 | ~ | ~ | 0 |
| 6 - Influent | | ~ | 0 | TNTC ($10^4$) | TNTC ($10^4$) | 0 | 0 | 0 |
| 1 - Effluent | | $6.3 \times 10^5$ | $1.3 \times 10^3$ | 0 | $1.0 \times 10^1$ | $1.3 \times 10^3$ | $1.3 \times 10^3$ | $2.5 \times 10^3$ |
| 2 - Effluent | | $2.4 \times 10^4$ | $6.0 \times 10^2$ | $1.0 \times 10^1$ | $1.0 \times 10^1$ | 0 | 0 | $1.6 \times 10^3$ |
| 3 - Effluent | | 0 | 0 | 0 | 0 | 0 | 0 | |
| 4 - Effluent | | $6.0 \times 10^4$ | $7.48 \times 10^6$ | $8.0 \times 10^6$ | $3 \times 10^1$ | $1.9 \times 10^6$ | $1.9 \times 10^6$ | $2.24 \times 10^6$ |
| 5 - Effluent | | 0 | 0 | 0 | 0 | 0 | 0 | |
| 6 - Effluent | | $1.0 \times 10^7$ | $6.02 \times 10^6$ | $1.8 \times 10^6$ | $1.27 \times 10^6$ | $1.7 \times 10^3$ | $1.7 \times 10^3$ | $5.1 \times 10^3$ |



WESTON

Soil column effluent viability was monitored as described in Subsection 3.5.2.2 with only limited variation observed. During the first 69 days of operation, columns 3 and 5 had markedly reduced effluent microbial counts, but were not completely sterile. At that time, the mercuric chloride level in the wastewater influent was increased from 0.50 percent to 0.75 percent. This resulted in complete inactivation, as illustrated by the enumerations reported in Table 4-7. Effluent samples from column 3 and 5 remained sterile for the remainder of the experiment.

Effluent samples from columns 1, 2, 4, and 6 were viable throughout the test. Column 2 had a period of low counts on days 217 through 249, but had a final count of $1.6 \times 10^3$ on day 260. This final count was of the same order of magnitude as corresponding samples from the other active columns.

4.1.14 Temperature. The continuous flow soil columns and perfusion columns were maintained at room temperature. During the nine months of the column operation, the mean temperature was 23°C with highest temperature recorded in the room at 26°C and lowest temperature recorded at 17°C. A complete temperature record is presented in Appendix I.

4.2 Soil perfusion column. Soil perfusion columns were utilized to determine if repeated passage through soil, or passage through a longer soil column (as would be the case in situ), would result in complete removal of wastewater components. Four soil perfusion columns were operated for 84 days.

SOIL PERFUSION COLUMNS

| No. | Medium | Microorganisms | Supplement |
|---|---|---|---|
| 1 | Tap water | Active | None |
| 2 | Wastewater | Active | None |
| 3 | Wastewater | Sterile | Glucose |
| 4 | Wastewater | Active | Whey |

The column reservoirs were sampled weekly for NQ as an indicator parameter. After 84 days of operation, no significant change in nitroguanidine or nitrosoguanidine levels were detected in any of the columns (Table 4-8). At this time a complete analysis for all components was performed. An obvious decrease in ammonia and an increase in nitrite-nitrate were

TABLE 4-8. SOIL PERFUSION COLUMN RESERVOIR CONCENTRATIONS
NQ AS INDICATOR

| Nitroguanidine mg/l | | | | | | Nitrosoguanidine mg/l | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | column | | | | | column | | | | |
| Day | 1 | 2 | 3 | 4 | Day | 1 | 2 | 3 | 4 |
| 0 | 0 | 129 | 129 | 129 | 0 | --- | --- | --- | --- |
| 7 | 92 | 130 | 130 | 110 | 7 | --- | --- | --- | --- |
| 15 | 11 | 123 | 132 | 119 | 15 | <0.02 | 0.40 | 0.40 | 0.30 |
| 21 | 6 | 135 | 136 | 72 | 21 | <0.02 | 0.50 | 0.40 | <0.02 |
| 29 | 13 | 169 | 163 | 183 | 29 | <0.02 | 0.90 | 1.08 | 0.92 |
| 35 | 16.9 | 117 | 190.6 | 194.6 | 35 | <0.20 | 0.37 | 0.36 | 0.35 |
| 43 | 10.8 | 115 | 103 | 195.6 | 43 | <0.20 | <0.20 | <0.20 | 0.40 |
| 84 | 4.8 | 72.0 | 79.9 | 79.9 | 84 | <0.20 | <0.20 | <0.20 | <0.20 |

detected. Total organic carbon (TOC) was reduced in all columns. No substantial changes occurred in sulfate levels and no intermediates were detected except cyanamide (Table 4-9).

4.3 Mineralization.

4.3.1 Mineralization rate potential. A mineralization rate potential experiment was conducted to obtain baseline data using $^{14}$C-glucose and a $^{14}$C-amino acid mixture. The SFAAP soil, as received, exhibited metabolic activity as determined by biodegradation of both glucose and amino acids. Approximately 30 percent of the $^{14}$C-glucose and $^{14}$C-amino acids initially added was recovered as $^{14}CO_2$ during 20 to 42 days of incubation at 20°C. (see Figures 4-5 and 4-6). Mineralization was rapid during the first seven days of incubation, followed by a slower mineralization rate over the remainder of the experiment. Similar percent recoveries were obtained for each of these substrates in three separate experiments using SFAAP soil as well as one experiment using West Chester, Pennsylvania garden soil.

4.3.2 Cometabolism - varied concentration of carbon supplement. In order to evaluate cometabolism of NQ and GN, several types of carbon supplements were added to SFAAP soil over a range of concentration (5 - 200 mg/l). The 20 mg/l glucose supplement was selected because this corresponds to the ratio of glucose to NQ that Kaplan and Kaplan (7) found to be effective for complete cometabolism of NQ in soil. The format for this experiment is as follows:

TEST SCHEME

| Additive | $^{14}$C-NQ | $^{14}$C-GN |
|---|---|---|
| Glucose 20 mg/l | 3 Replicates | 3 |
| Whey 200 | 3 | 3 |
| 100 | 3 | 3 |
| 20 | 3 | 3 |
| 5 | 3 | 3 |
| Molasses 200 | 3 | 3 |
| 100 | 3 | 3 |
| 20 | 3 | 3 |
| 5 | 3 | 3 |
| Unsupplemented | 3 | 3 |
| Glucose 20 mg/l Sterile | 3 | 3 |

TABLE 4-9. SOIL PERFUSION COLUMN RESERVOIR CONCENTRATIONS

| Compound | mg/l | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | | | | Day 84 | | | |
| | Column | | | | Column | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Nitroguanidine (NQ) | <0.2 | 129 | 129 | 129 | 4.8 | 72.0 | 79.9 | 79.9 |
| Cyanamide (C) | <2.0 | <2.0 | <2.0 | <2.0 | <2.0 | 6.4 | 7.4 | 5.9 |
| Cyanoguanidine | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| Nitrosoguanidine (NOQ) | --- | --- | --- | --- | <0.2 | <0.2 | <0.2 | <0.2 |
| Melamine (M) | --- | --- | --- | --- | <2.0 | <2.0 | <2.0 | <2.0 |
| Guanidine Nitrate | --- | 10.5 | 10.5 | 10.5 | <5.0 | <5.0 | <5.0 | <5.0 |
| Guanidine (G) | --- | --- | --- | --- | --- | --- | --- | --- |
| Nitrite-Nitrate ($NO_2$-$NO_3$) | --- | 11.0 | 11.0 | 11.0 | 10.8 | 30.8 | 58.8 | 46.5 |
| Ammonia Nitrogen ($NH_3$-N) | --- | 25 | 25 | 25 | <0.3 | <0.3 | <0.3 | <0.3 |
| Sulfate ($SO_4$) | --- | 120 | 120 | 120 | 24 | 107 | 140 | 118 |
| Total Organic Carbon (TOC) | <1.0 | <1.0 | 1,000 | 1,000 | 6.14 | 12.5 | 13.5 | 13.1 |
| Ammonia Nitrate | 0 | 12.5 | 12.5 | 12.5 | --- | --- | --- | --- |
| Sodium Sulfate | 0 | 166.25 | 166.25 | 166.25 | --- | --- | --- | --- |

Figure 4-5

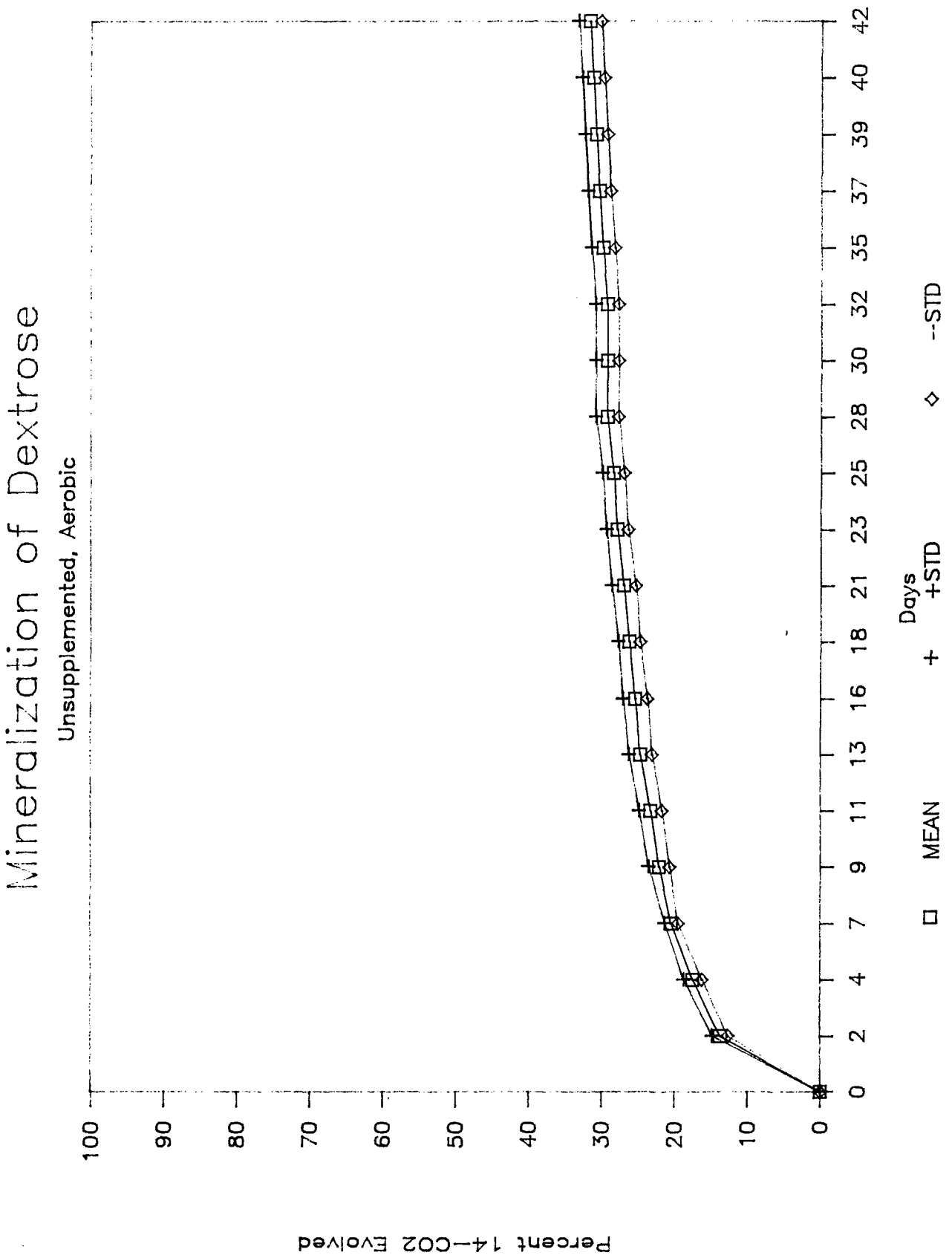
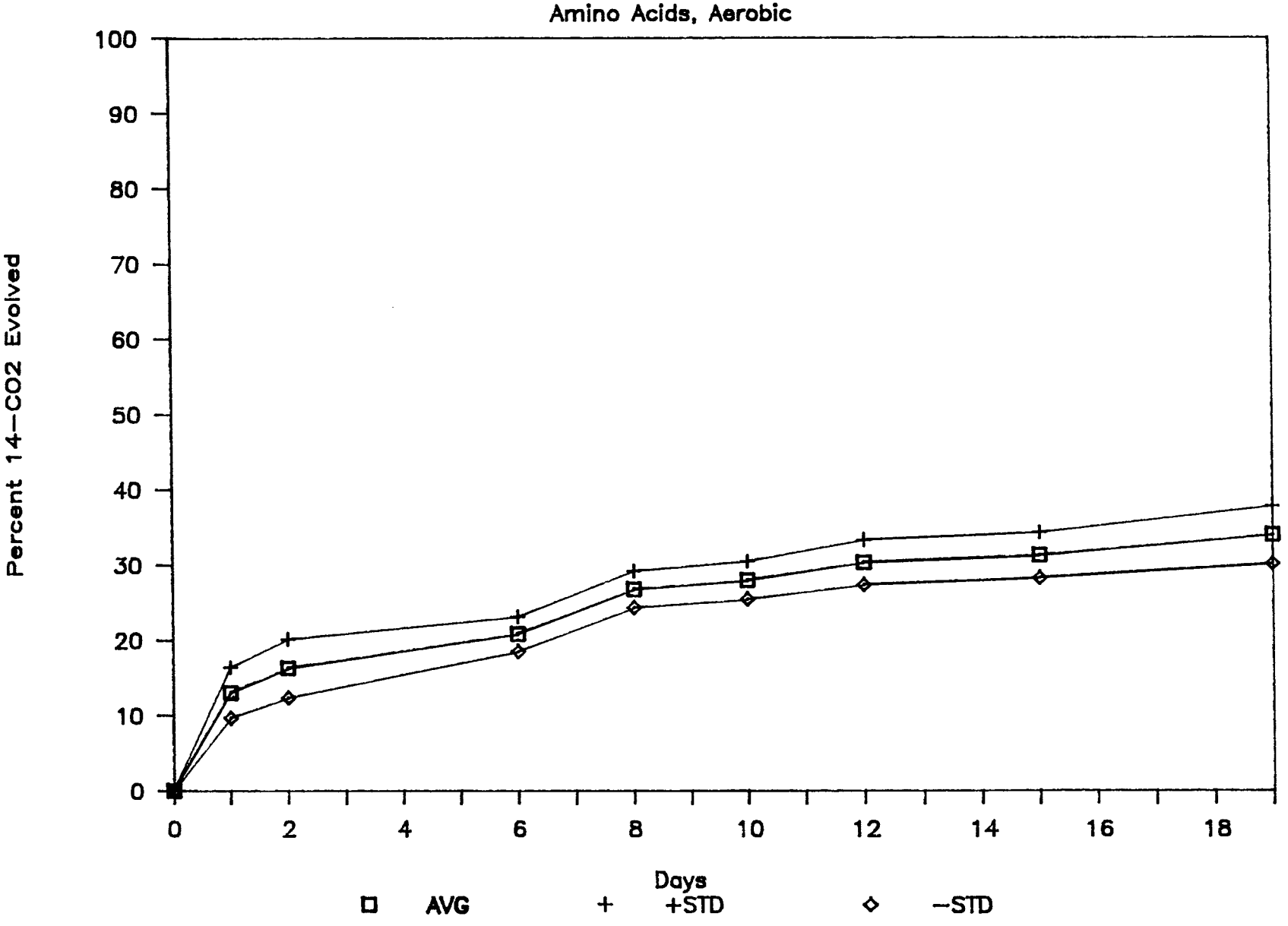

Mineralization of Dextrose
Unsupplemented, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0 2 4 7 9 11 13 16 18 21 23 25 28 30 32 35 37 39 40 42
Days
MEAN
+STD
--STD

Figure 4-6

# Mineralization Potential of SFAAP Soil



Test flasks were innoculated with acclimated microorganisms from an acclimated soil column and activated sludge. Nutrients and other additives were provided as described in Section 3. The soil was incubated in flasks under aerobic conditions at 20°C and test flasks maintained until $^{14}CO_2$ evolution reached a plateau.

Nitroguanidine mineralization was low, with less than 15 percent of the $^{14}$C-NQ added being evolved as $^{14}CO_2$ under all experimental conditions, except when molasses was provided at 200 mg/l (50 ±28 percent). The relatively high (50 percent) mineralization of NQ in the presence of 200 mg/l molasses is most likely incorrect, based on the relative ineffectiveness of other organic supplements and the lack of a dose-response correlation in the case of molasses. As the shape of the $^{14}CO_2$-evolution curve looks reasonable, a dosing error seems to be the most likely explanation. All other factors indicate that even with 200 mg/l molasses, the true mineralization of NQ is less than 20 percent. Data from this experiment are presented in Table 4-10.

The majority of $^{14}CO_2$ was evolved during days 10 to 30. A typical nitroguanidine mineralization curve is shown in Figure 4-7. A complete graphic presentation of mineralization data from this experiment is found in Appendix J.

Guanidine nitrate was rapidly and extensively mineralized. Greater than 50 percent of the $^{14}$C-GN added was evolved as $^{14}CO_2$ (Table 4-11) under all active test conditions with the majority of this transformation occurring in the first 48 hours of incubation. A typical guanidine nitrate mineralization curve is shown in Figure 4-8.

4.3.3 Mineralization of NQ and GN under aerobic and anaerobic conditions. The mineralization of NQ and GN under aerobic and anaerobic conditions was investigated in SFAAP soil supplemented with 100 mg/l carbon (provided as glucose, whey, or molasses). The following experimental scheme was used:

TEST SCHEME

| Supplement | Aerobic | | Anaerobic | |
|---|---|---|---|---|
| | $^{14}$C-NQ | $^{14}$C-GN | $^{14}$C-NQ | $^{14}$C-GN |
| Glucose (100 mg/l) | 3 | 3 | 3 | 3 |
| Whey (100 mg/l) | 3 | 3 | 3 | 3 |
| Molasses (100 mg/l) | 3 | 3 | 3 | 3 |
| Unsupplemented | 3 | 3 | 3 | 3 |
| Sterile | 3 | 3 | 3 | 3 |

TABLE 4-10. MINERALIZATION OF NQ IN SFAAP SOIL

| Carbon supplement | mg/l | | Aerobic<br>Percent $^{14}CO_2$ evolution |
|---|---|---|---|
| Glucose | 20 | | 14 ± 3.0 |
| Molasses | 5 | | 10 ± 0.7 |
| Molasses | 20 | | 10 ± 2.0 |
| Molasses | 100 | | 8.0 ± 0.2 |
| Molasses | 200 | | 50 ± 28 |
| Whey | 5 | | 12 ± 2.0 |
| Whey | 20 | | 11 ± 2.5 |
| Whey | 100 | | 11 ± 0.5 |
| Whey | 200 | | 13 ± 1.0 |
| Unsupplemented | | | 11 ± 1.0 |
| Glucose | 20 | sterile | 0.4 ± 0.2 |

Inoculum = Extract of acclimated soil and activated sludge.

TABLE 4-11. MINERALIZATION OF GN IN SFAAP SOIL

| Carbon supplement | mg/l | | Aerobic<br>Percent $^{14}CO_2$ evolution |
|---|---|---|---|
| Glucose | 20 | | 49 ± 11 |
| Molasses | 5 | | 81 ± 18 |
| Molasses | 20 | | 75 ± 10 |
| Molasses | 100 | | 66 ± 5 |
| Molasses | 200 | | 81 ± 22 |
| Whey | 5 | | 40 ± 20 |
| Whey | 20 | | 56 ± 27 |
| Whey | 100 | | 79 ± 10 |
| Whey | 200 | | 81 ± 8 |
| Unsupplemented | | | 98 ± 15 |
| Glucose | 20 | sterile | 0.5 ± 0.06 |

Inoculum = Extract of acclimated soil and activated sludge.

Figure 4-7

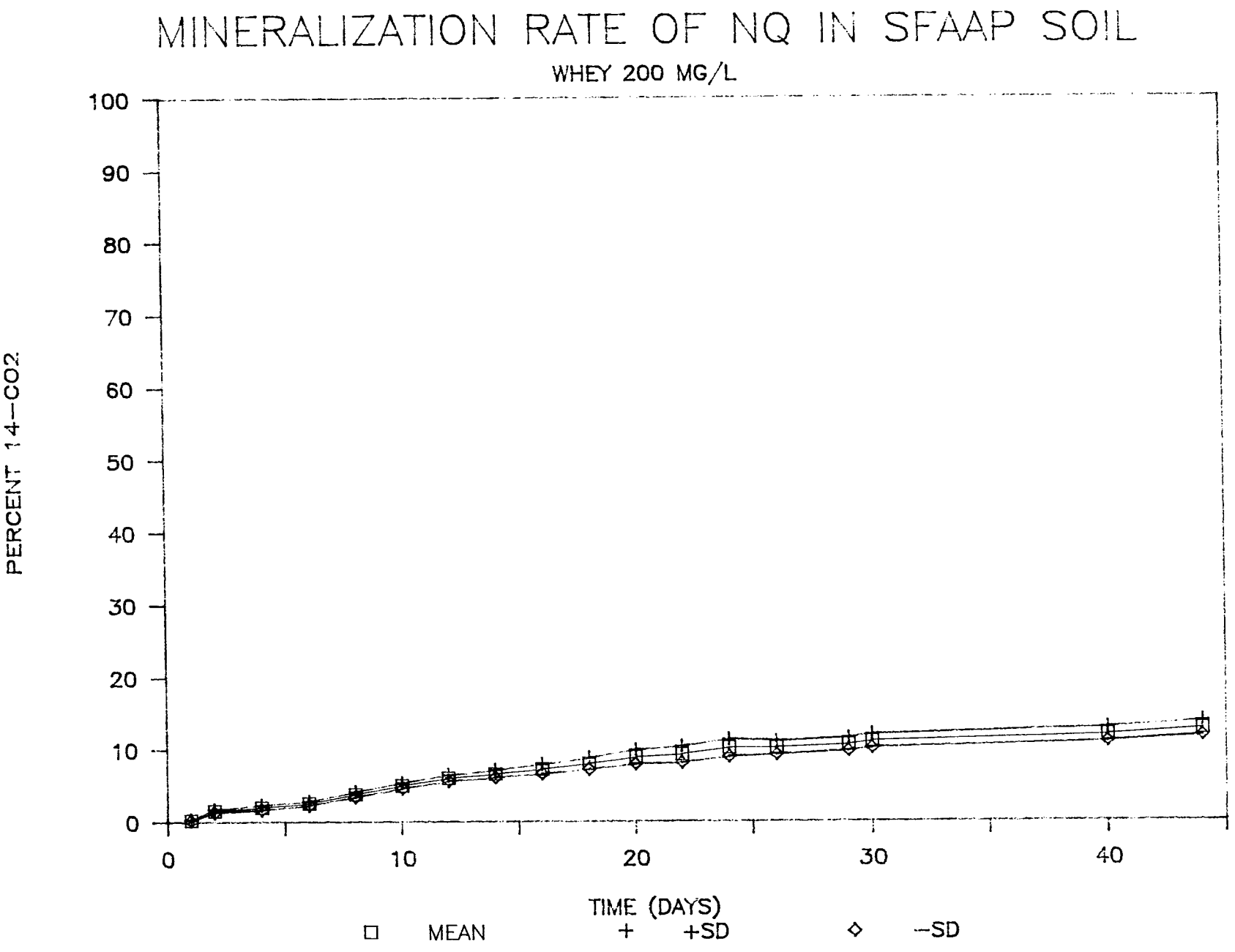
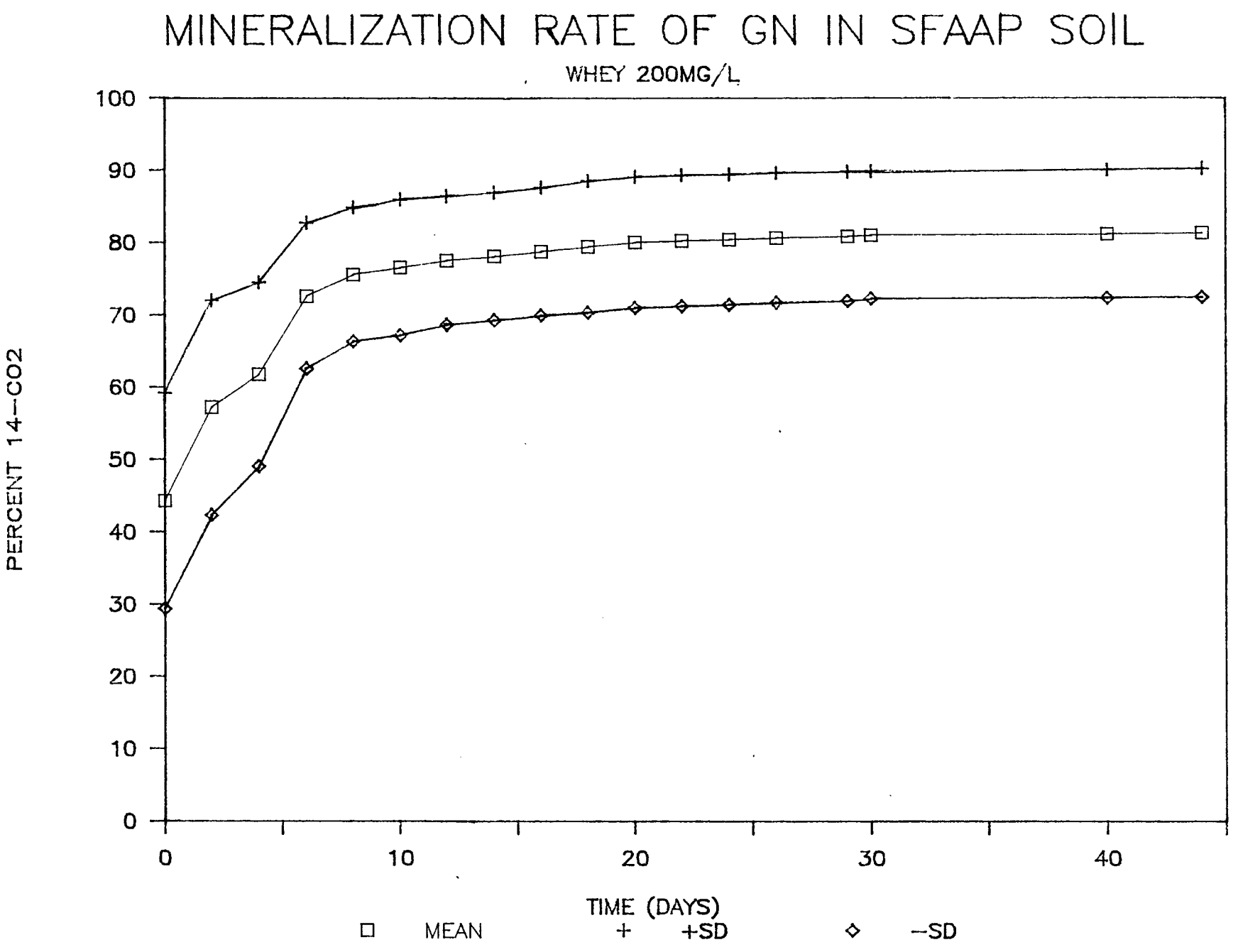

MINERALIZATION RATE OF NQ IN SFAAP SOIL
WHEY 200 MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

Figure 4-3


MINERALIZATION RATE OF GN IN SFAAP SOIL
WHEY 200MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

Less than 11 percent of the $^{14}C$-nitroguanidine added to the soil was evolved as $^{14}CO_2$ after 44 days incubation under either aerobic or anaerobic conditions. The sterile control evolved less than 10 percent of the $^{14}CO_2$ evolved from active flasks. Carbon supplements did not increase the mineralization rate of NQ (Table 4-12). Illustrative nitroguanidine mineralization data are presented in Figures 4-9 and 4-10, and the complete set of NQ mineralization curves for this experiment is presented in Appendix K.

Greater than 50 percent of the $^{14}C$-guanidine nitrate added to the soil was evolved as $^{14}CO_2$ after 44 days incubation under aerobic conditions at 20°C (Table 4-13). Rapid mineralization occurred during the first 48 hours with slower mineralization occurring from day 2 to day 9. The evolution of $^{14}CO_2$ reached a plateau during days 9 through 44 of incubation. A typical aerobic mineralization curve of guanidine nitrate is shown in Figure 4-11.

Under anaerobic conditions at room temperature, between 40 to 55 percent of the $^{14}C$-GN added to the soil was evolved as $^{14}CO_2$ (Table 4-13). A typical anaerobic mineralization curve of guanidine nitrate is shown in Figure 4-12. Graphs of GN mineralization in pretreatment soil under aerobic and anaerobic conditions are presented in Appendix L. Addition of carbon supplements glucose, whey, or molasses had no significant impact on the mineralization of GN under aerobic or anaerobic conditions.

The greater variation observed under anaerobic conditions probably reflects differences in microbial activity within an experimental set rather than difficulties with the experimental apparatus or methods.

4.3.4 Volatility check of guanidine nitrate. Two observations from prior experiments raised concern that what was considered to be $^{14}CO_2$ from $^{14}C$-GN mineralization, may in fact have been either $^{14}C$-GN or a volatile intermediate. These observations were first, the high percentage of added $^{14}C$ recovered as $^{14}CO_2$ and second, the rate at which the $^{14}CO_2$ was recovered. Volatility was not expected to be a concern, but the data indicated that the trapped material should be confirmed to be $CO_2$.

Three tests were performed to evaluate this concern:

(a) Mineralizaton of guanidine nitrate using an apparatus designed for monitoring the mineralization of volatile $^{14}C$-labeled compounds.
(b) An alkali trapping procedure to separate $CO_2$ from organics. This test was used for both NQ and GN.

TABLE 4-12. MINERALIZATION OF NQ IN SFAAP SOIL

| Carbon supplement 100 mg/l | Inoculum | Percent $^{14}CO_2$ evolved | |
|---|---|---|---|
| | | Aerobic | Anaerobic |
| Glucose | Acclimated Bacteria | 6.1± 2.9 | 2.6± 2.29 |
| Whey | Acclimated Bacteria | 6.6± 3.7 | 5.4± 2.75 |
| Molasses | Acclimated Bacteria | 6.4± 2.9 | 3.19± 1.65 |
| None | Acclimated Bacteria | 5.7± 0.4 | 10.7± 5.7 |
| Glucose | Sterile | 0.4± 0.2 | 0.22± 0.13 |

TABLE 4-13. MINERALIZATION OF GN IN SFAAP SOIL

| Carbon Supplement 100 mg/l | Innoculation | Percent $^{14}CO_2$ evolved | | | |
|---|---|---|---|---|---|
| | | Aerobic Mean | * | Anaerobic Mean | * |
| Glucose | Acclimited bacteria | 60 | ±6 | 41.6 | ±35 |
| Whey | Acclimated bacteria | 59 | ±10 | 41.6 | ±21 |
| Molasses | Acclimated bacteria | 75 | ±20 | 48 | ±19 |
| None | Acclimated bacteria | 98 | ±13 | 58 | ±15 |
| Glucose | Sterile | 2 | ±0.2 | 9.0 | ±6 |

*Standard deviation

Figure 4-9

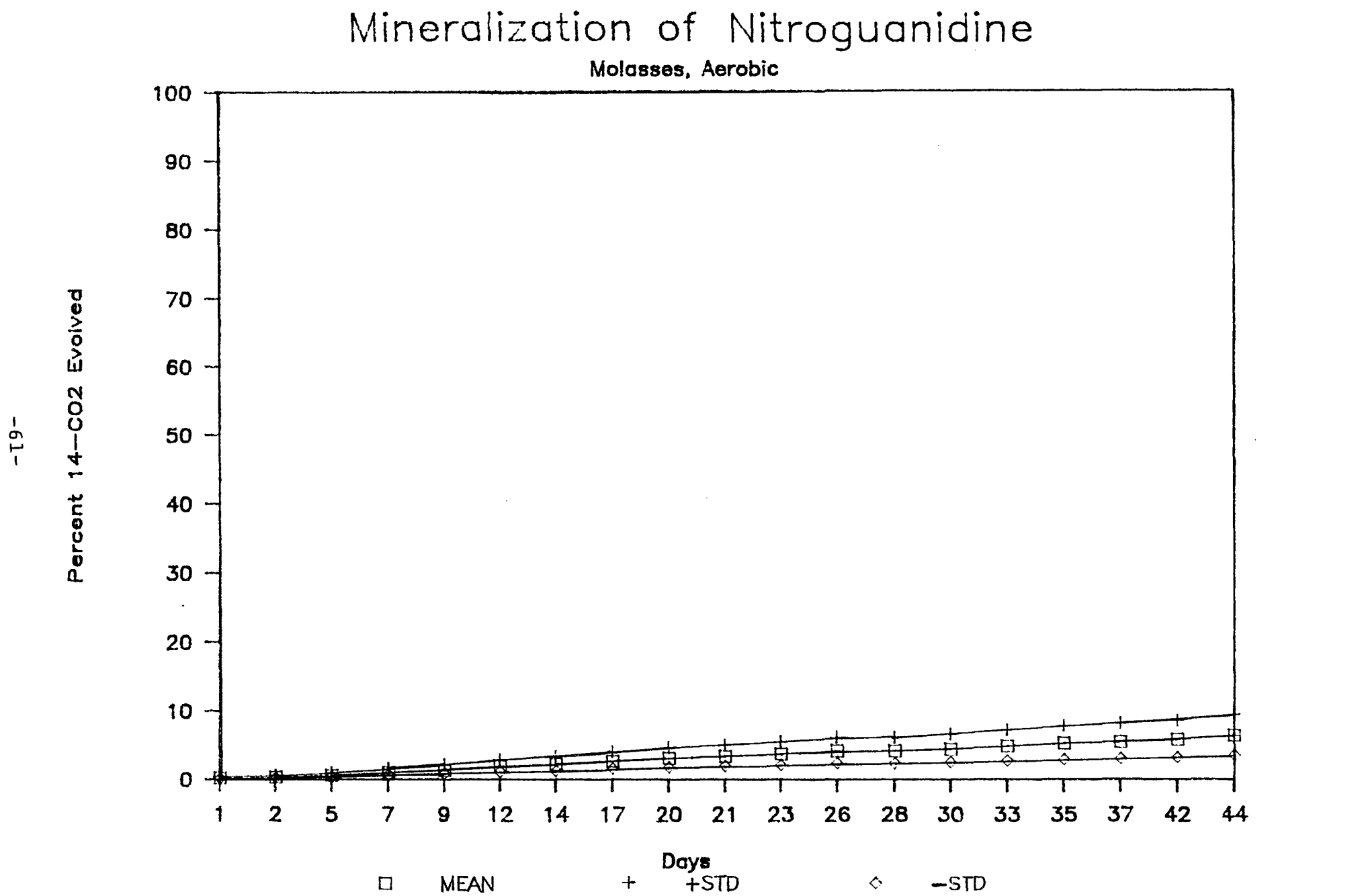

Mineralization of Nitroguanidine
Molasses, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
17
20
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
—STD

Figure 4-10

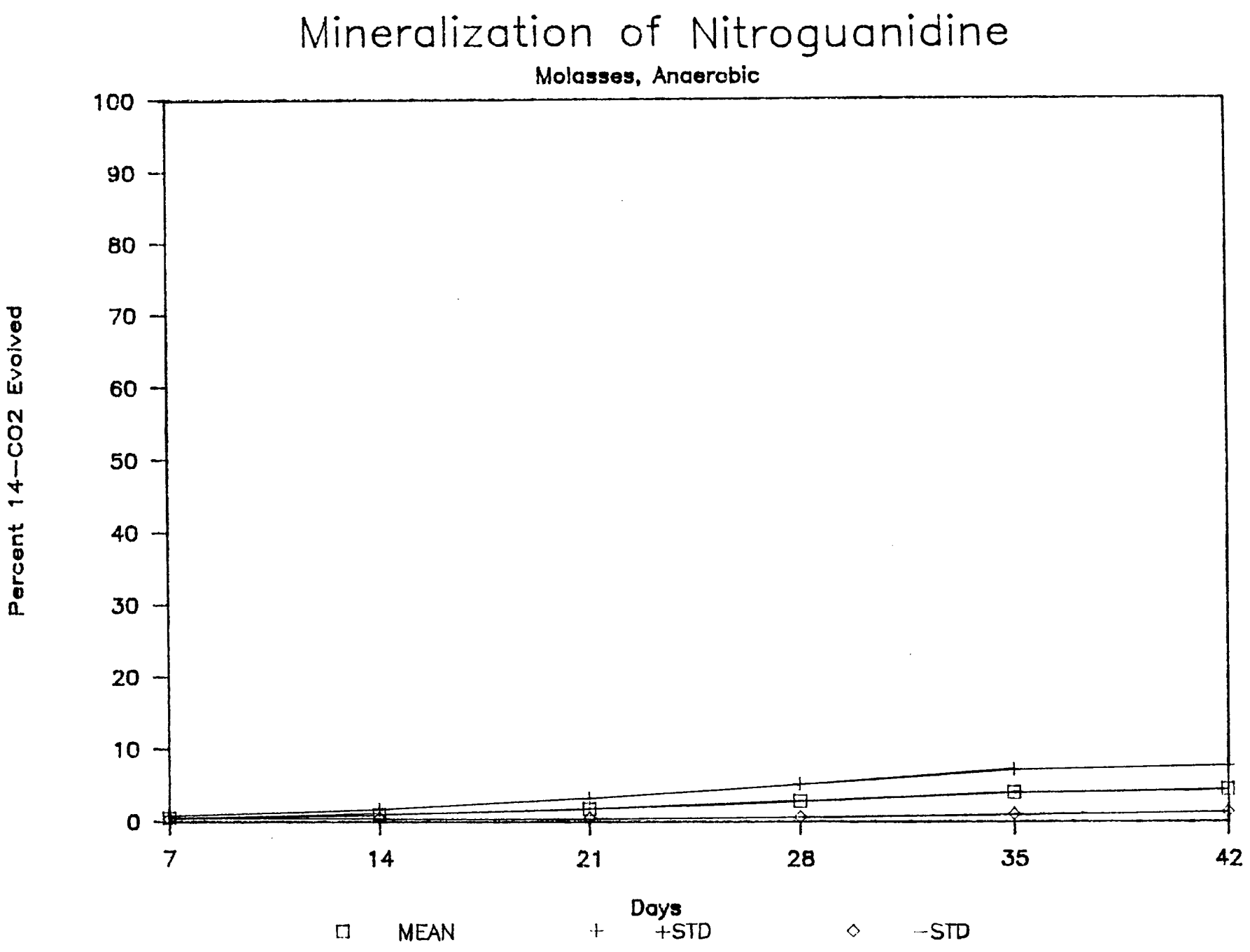

Mineralization of Nitroguanidine
Molasses, Anaerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
-STD

Figure 4-11

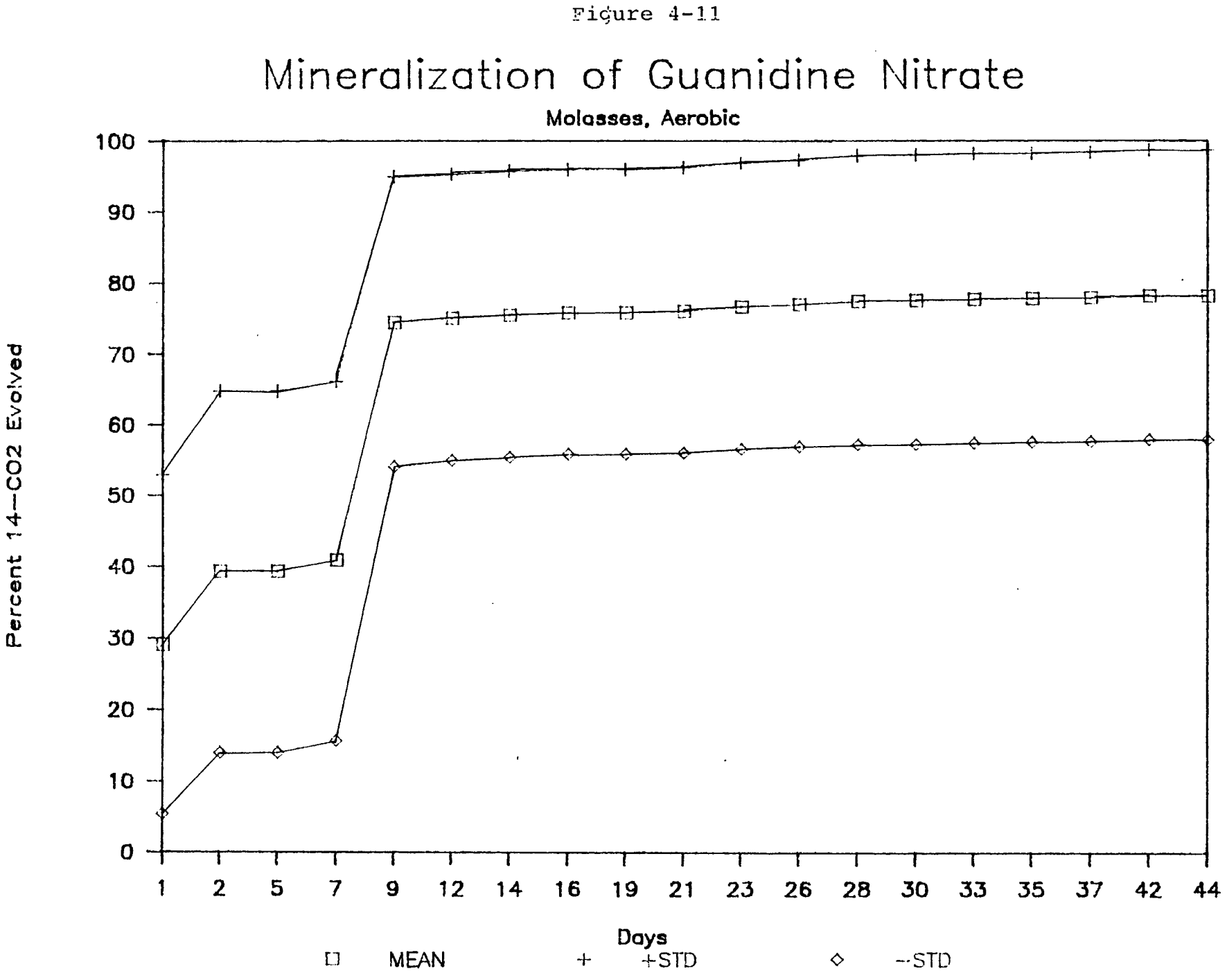
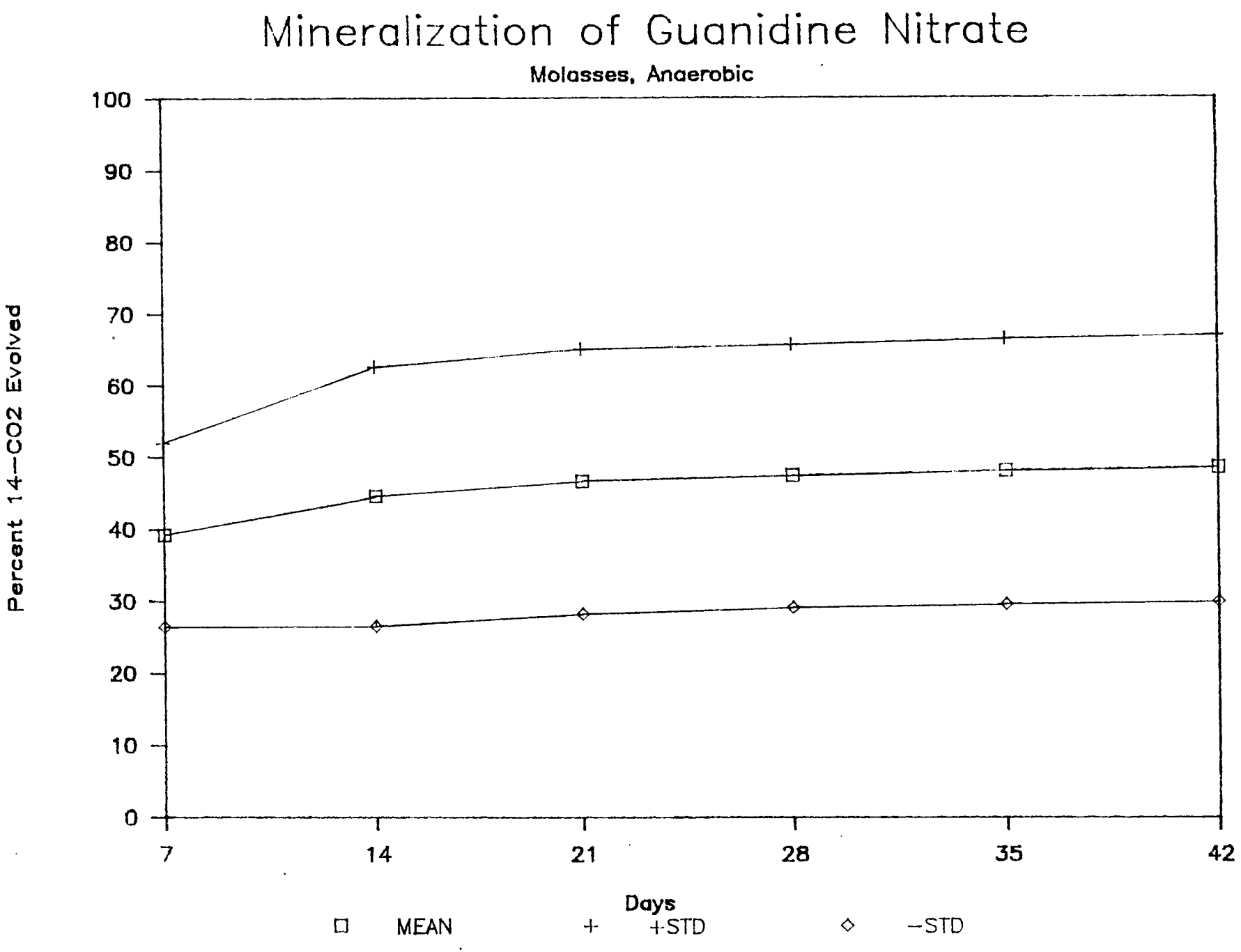

Mineralization of Guanidine Nitrate
Molasses, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
-STD

Figure 4-12


Mineralization of Guanidine Nitrate
Molasses, Anaerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
-STD

(c) A swab test for compound adsorption to exposed surfaces.

All three of these tests are described in detail in Subsection 3.7.

Using the volatile compound mineralization apparatus, less than 2 percent of the $^{14}C$-guanidine nitrate was recovered in the volatile organic trapping solution after 44 days of incubation under either aerobic or anaerobic conditions and either with or without carbon supplements (glucose, molasses, or whey). Active soil and sterile soil gave similar results (Table 4-14). A typical curve of guanidine nitrate volatility is shown in Figure 4-13. See appendix M for graphs of GN volatilization.

The alkali trap (precipitation) method was used to test the chemical nature of the $^{14}C$ evolved from experiments using $^{14}C$-GN and $^{14}C$-NQ. All the $^{14}C$ recovered from $^{14}C$-NQ mineralization experiments and greater than 96 percent of the $^{14}C$ recovered from $^{14}C$-GN experiments proved to be $^{14}CO_2$. The alkali trap test was conducted using unsterilized SFAAP soil. A soil test was also conducted to test for adsorption of $^{14}C$ guanidine nitrate to exposed surfaces in the test flask. The dpm detected on all tested surfaces was at or below background (30.5 dpm).

4.3.5 Short-term characterization of GN mineralization. In order to characterize the mineralization rate of guanidine nitrate, hourly purges of mineralization flasks containing $^{14}C$ guanidine nitrate were conducted during the first 48 hours of incubation. Based on percent evolved $^{14}CO_2$ from parent compound, the mineralization rate of guanidine nitrate was 1.2 percent/hour during the first 48 hours of incubation (Table 4-15). After 48 hours, the mineralization rate slowed to 0.19 percent/hour for the remaining 52 hours of the study. Rates were calculated by averaging the evolved $^{14}CO_2$ over time of incubation. The rates did not remain linear within these two periods. The incubation temperature was 20± 2°C, and the flasks were aerobic. A graphic presentation of the hourly mineralization rate of guanidine nitrate is presented in Figure 4-17.

TABLE 4-14. VOLATILIZATION OF GUANIDINE NITRATE

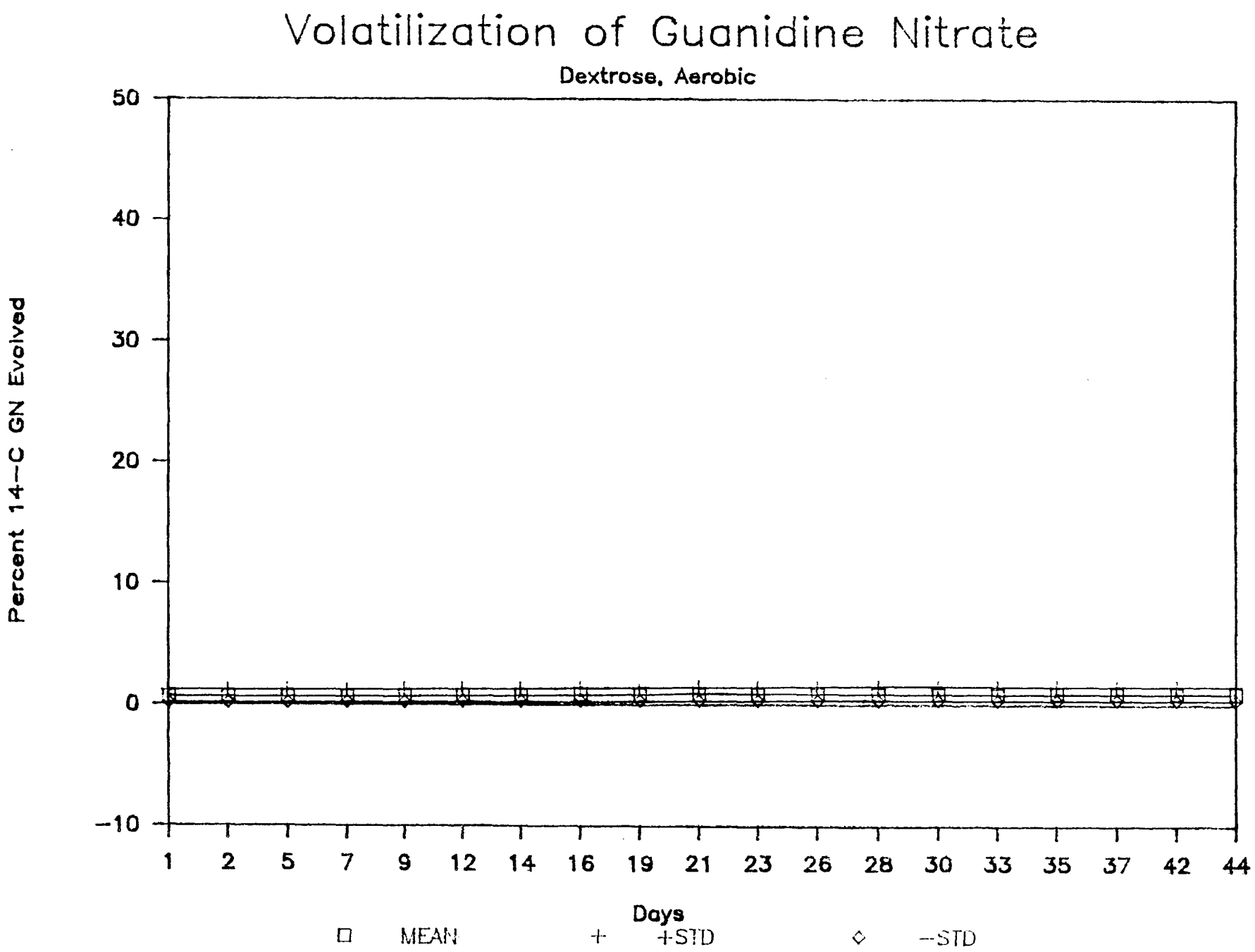
| Carbon supplement | Microbial condition | Percent $^{14}C$ recovered Aerobic Mean | Aerobic Std* | Anaerobic Mean | Anaerobic Std* |
|---|---|---|---|---|---|
| Glucose | Active | 1.06 | ±0.49 | 0.13 | ±0.03 |
| Molasses | Active | 1.93 | ±1.07 | 0.23 | ±0.12 |
| Whey | Active | 0.5 | ±0.18 | 2.5 | ±0.3 |
| None | Sterile | 0.06 | ±0.06 | 0.05 | ±0.2 |

*Standard Deviation
Time course data are presented graphically in Appendix M.

TABLE 4-15. HOURLY MINERALIZATION RATE OF GUANIDINE NITRATE IN SFAAP SOIL

| Inoculum | Rate (% $^{14}CO_2$/hr) Time period (hours) 0-48 | 48-100 |
|---|---|---|
| Native Microflora | 1.1 | 0.14 |
| Soil and Activated Sludge | 1.1 | 0.17 |
| Soil and Acclimated Bacteria | 1.4 | 0.27 |

Figure 4-13


Volatilization of Guanidine Nitrate
Dextrose, Aerobic
Percent 14-C GN Evolved
50
40
30
20
10
0
-10
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
-STD

4.3.6 Microbial adaptation. An experiment was conducted to determine if microorganisms exposed to NQ and GN were able to transform these compounds at a higher rate than unexposed organisms. The NQ and GN mineralization rate was compared in uninoculated SFAAP soil and in SFAAP soil inoculated with either activated sludge or acclimated bacteria. The soil samples were supplemented with nutrient solution, wastewater, and whey. Mineralization of NQ remained low (<11 percent $^{14}CO_2$) regardless of the microorganisms present (Table 4-16). The $^{14}CO_2$ evolution curves for all three inocula were essentially identical. A typical nitroguanidine mineralization curve is shown in Figure 4-14.

A slightly higher GN mineralization rate was observed with the addition of either acclimated bacteria (102 ±26 percent $^{14}CO_2$) or activated sludge (80 ±2.0 percent $^{14}CO_2$) to the soil compared to the native microflora (76 ±3.5 percent $^{14}CO_2$). The GN mineralization data are presented in Figures 4-15 through 4-18.

4.3.7 Mineralization in post-treatment soil. Acclimation of soil bacteria to wastewater components during the continuous flow soil column study was evaluated by retesting the mineralization of nitroguanidine, guanidine nitrate, and glucose simulated in SFAAP soil. This soil had been exposed to the wastewater for 271 days.

Nitroguanidine mineralization rates in post-treatment soil were similar to those in untreated soil, low under both aerobic or anaerobic conditions in soil from each of the six continuous flow soil columns. These rates were measured as evolved $^{14}CO_2$ from added $^{14}C$-nitroguanidine and ranged in soil from active columns from 0.4 to 0.8 percent under anaerobic conditions and from 2.4 to 10.3 percent under aerobic conditions (Table 4-17). A typical graph of nitroguanidine mineralization in post treatment soil is shown in Figure 4-19. A complete data set for NQ mineralization is included in Appendix N.

Guanidine nitrate mineralization rates in post-treatment soil were essentially the same as in untreated soil under aerobic and anaerobic conditions. Evolved $^{14}CO_2$ from added $^{14}C$-guanidine nitrate ranged from 60.3 to 90.3 percent in soil from active columns under aerobic conditions and from 44.9 to 59.9 percent in soil from active columns under anaerobic conditions (Table 4-17). A typical graph of guanidine nitrate mineralization rate in post-treatment soil is shown in Figure 4-20. GN mineralization curves for all columns are shown in Appendix O.

TABLE 4-16. BACTERIAL ACCLIMATION AND MINERALIZATION OF GUANIDINE NITRATE AND NITROGUANIDINE

| Medium | Inoculum | Aerobic Percent $^{14}CO_2$ evolved NQ | GN |
|---|---|---|---|
| SFAAP Soil | Native Microflora | ----- | 76.4± 3.5 |
| SFAAP Soil | Activated Sludge | 11± 0.6 | 80.3± 2.0 |
| SFAAP Soil | Acclimated Bacteria | 6.6± 3.7 | 102.0± 26.0 |
| SFAAP Soil | Sterile | 0.4± 0.2 | 0.6± 0.06 |

TABLE 4-17. MINERALIZATION IN POST-TREATMENT SFAAP SOIL
TOTAL PERCENT $^{14}CO_2$ EVOLVED

| | NQ | | GN | | Glucose | |
|---|---|---|---|---|---|---|
| | Aerobic | Anaerobic | Aerobic | Anaerobic | Aerobic | Anaerobic |
| Column 1 Active | 5.8± 0.5 | 0.8± 0.3 | 63.6± 16.5 | 53.3± 6.3 | 28.9± 4.6 | 19.0± 10.5 |
| Column 2 Active | 8.3± 1.5 | 0.4± 0.1 | 65.0± 7.7 | 49.2± 20.2 | 18.5± 4.0 | 23.6± 6.8 |
| Column 3 Sterile | 0.8± 0.3 | 0.0± 0.0 | 0.1± 0.0 | 0.2± 0.1 | 2.0± 0.85 | 0.79± 0.24 |
| Column 4 Active | 2.4± 0.2 | 0.6± 0.3 | 60.3± 7.5 | 59.9± 25.2 | 43.8± 13.5 | 50.0± 2.9 |
| Column 5 Sterile | 0.9± 0.1 | 0.1± 0.1 | 0.1± 0.0 | 0.97± 0.5 | 2.58± 0.46 | 2.2± 0.4 |
| Column 6 Active | 10.3± 7.1 | 0.7± 0.2 | 90.3± 29.2 | 44.9± 19.9 | 44.4± 4.21 | 43.5± 6.1 |

Values reported are the final percentage of added $^{14}C$ trapped as $^{14}CO_2$, plus/minus the standard deviation.
Time course data are presented graphically in Appendix N - NQ; Appendix O - GN; Appendix P - Glucose.

Figure 4-14

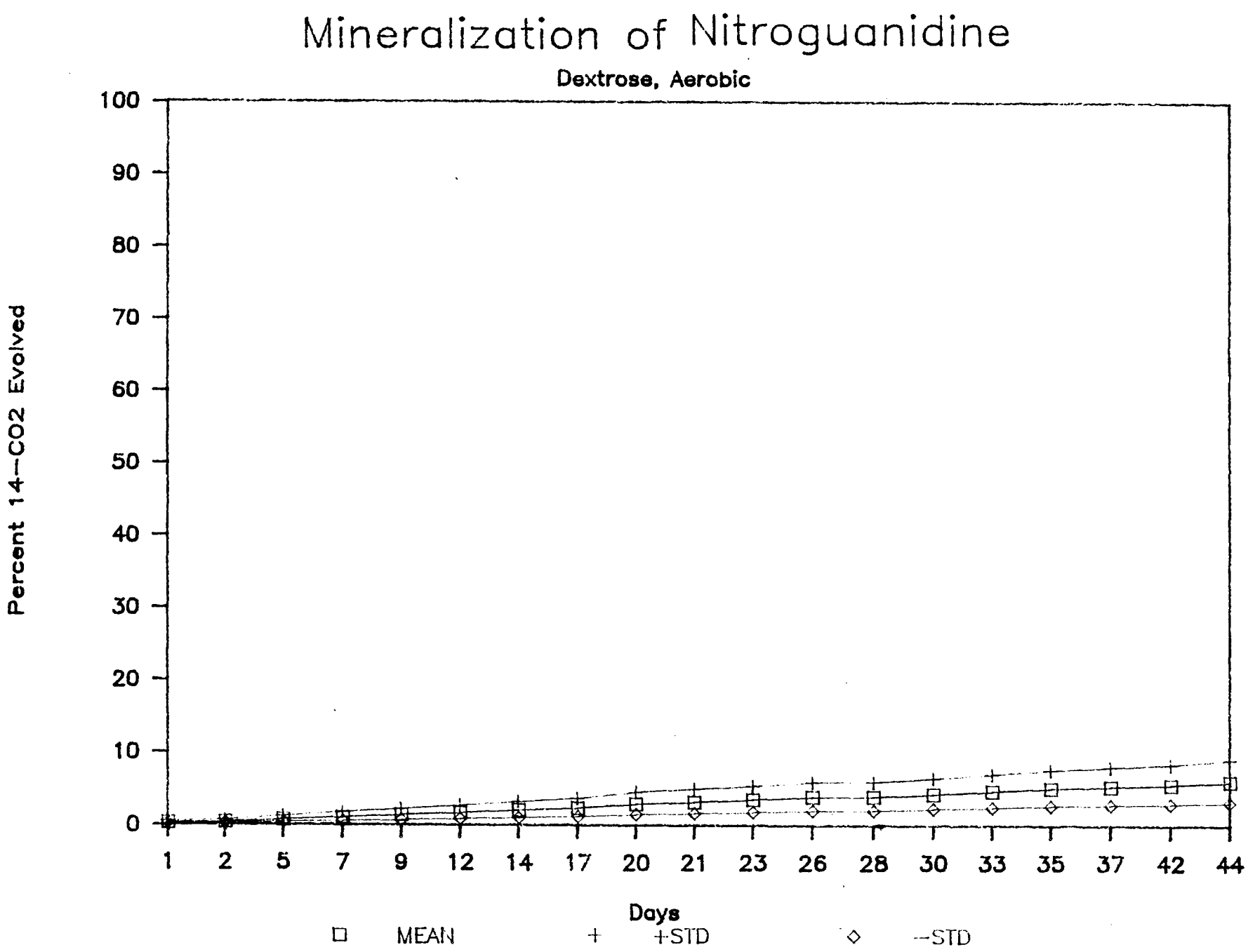

Mineralization of Nitroguanidine
Dextrose, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
17
20
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
—STD

Figure 4-15

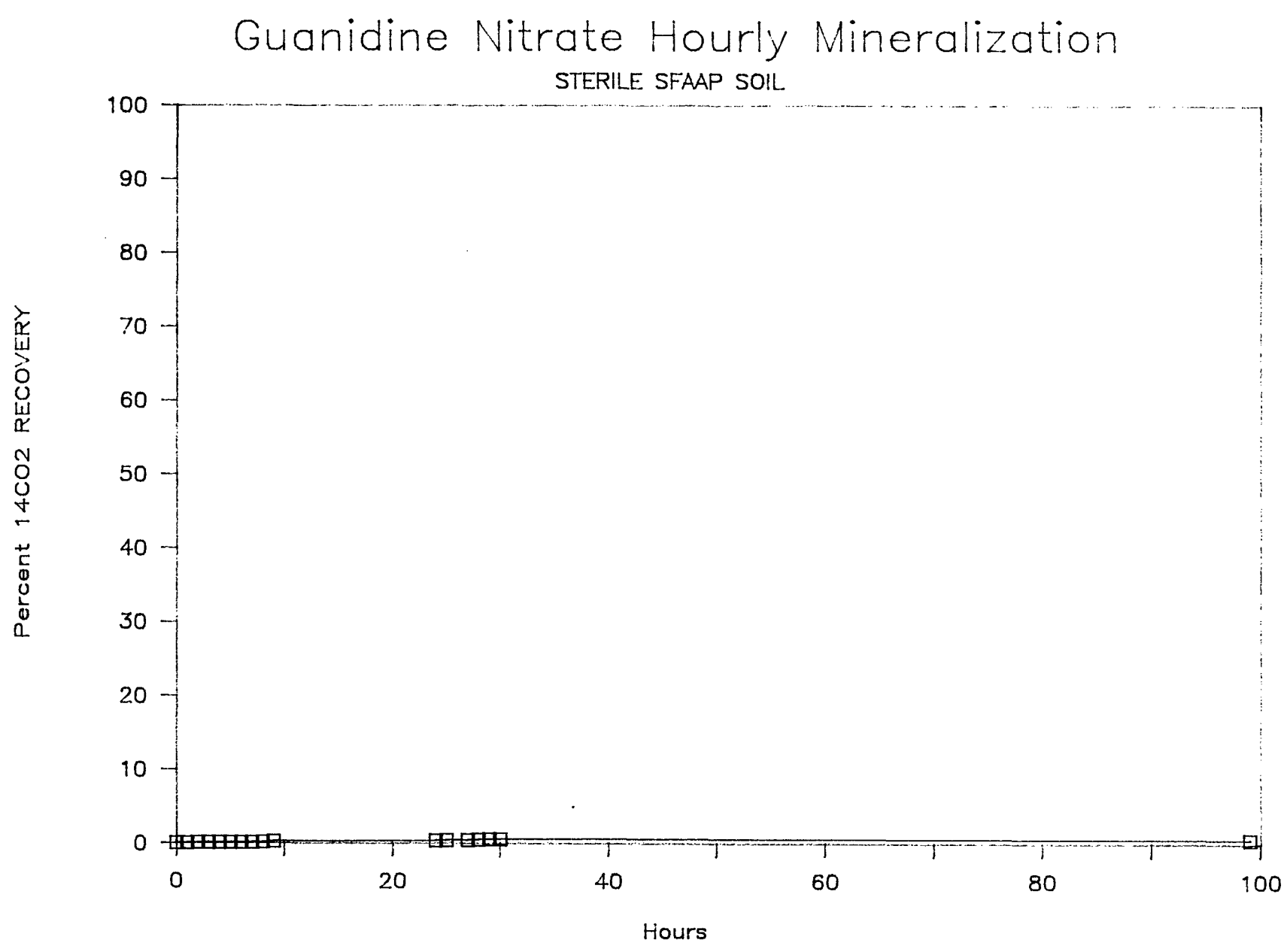
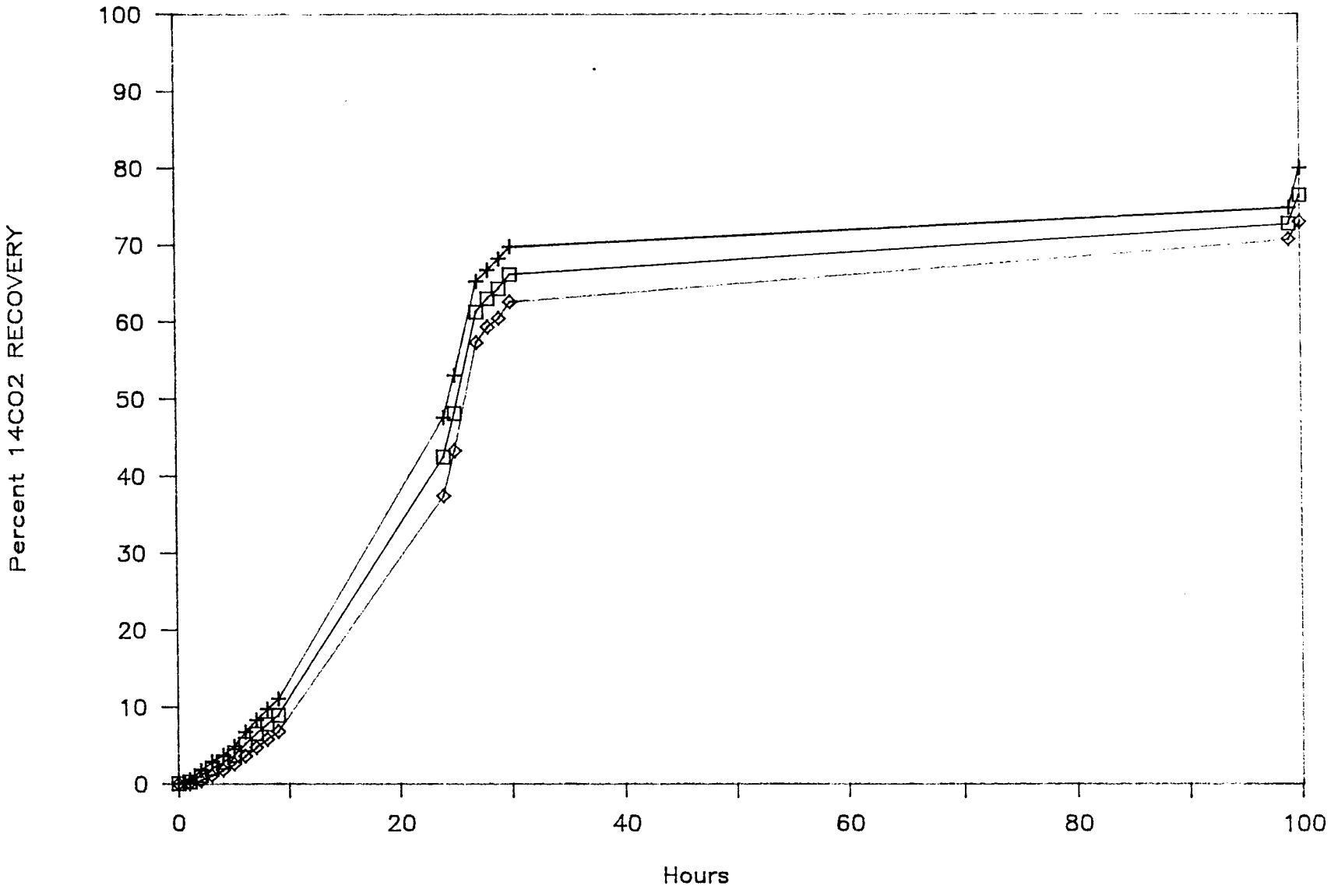

Guanidine Nitrate Hourly Mineralization
STERILE SFAAP SOIL
Percent 14CO2 RECOVERY
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Hours

Figure 4-10

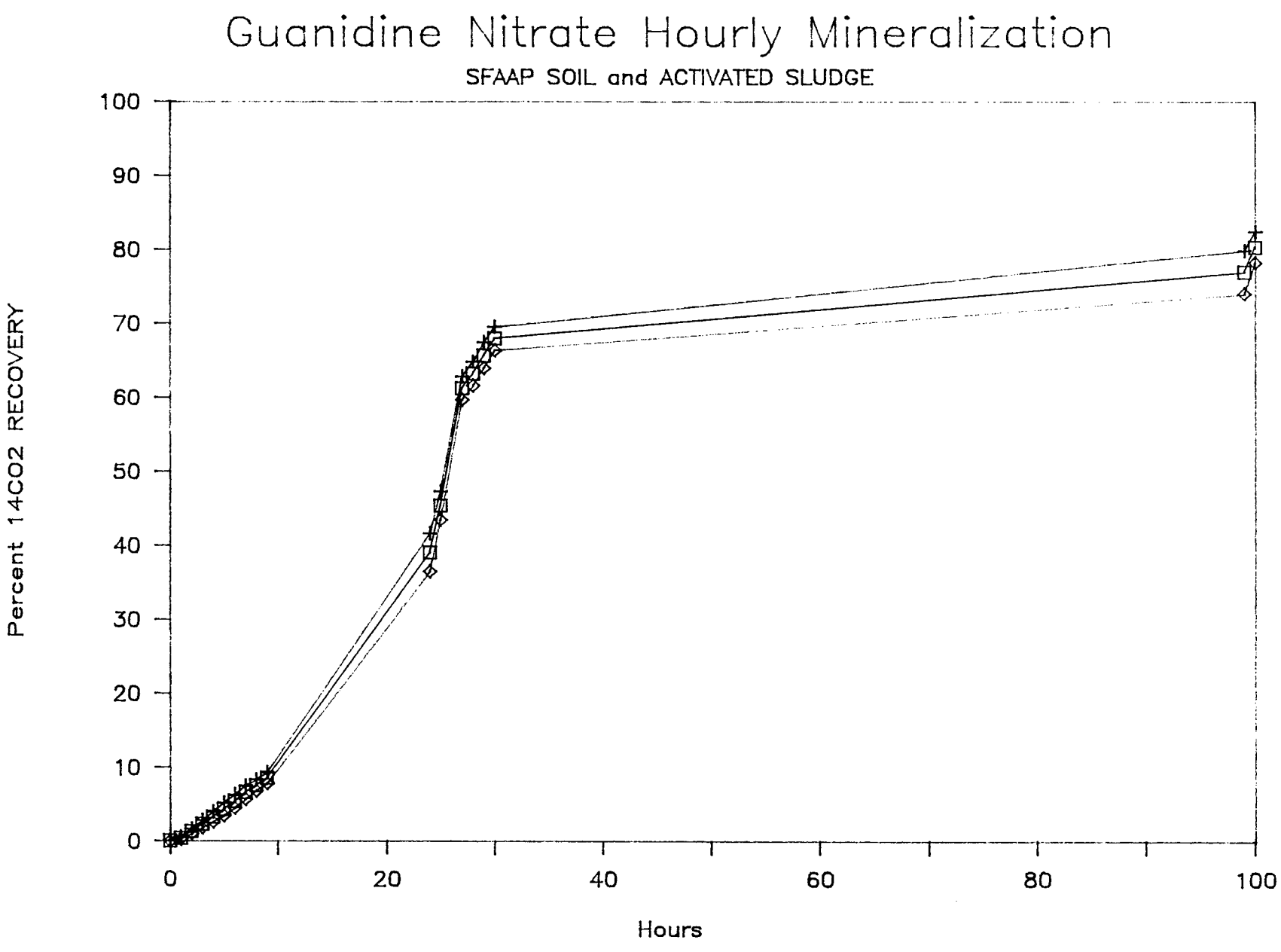
# Guanidine Nitrate Hourly Mineralization

SFAAP SOIL NATIVE FLORA




WESTON

Figure 4-17


Guanidine Nitrate Hourly Mineralization
SFAAP SOIL and ACTIVATED SLUDGE
Percent 14CO2 RECOVERY
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Hours

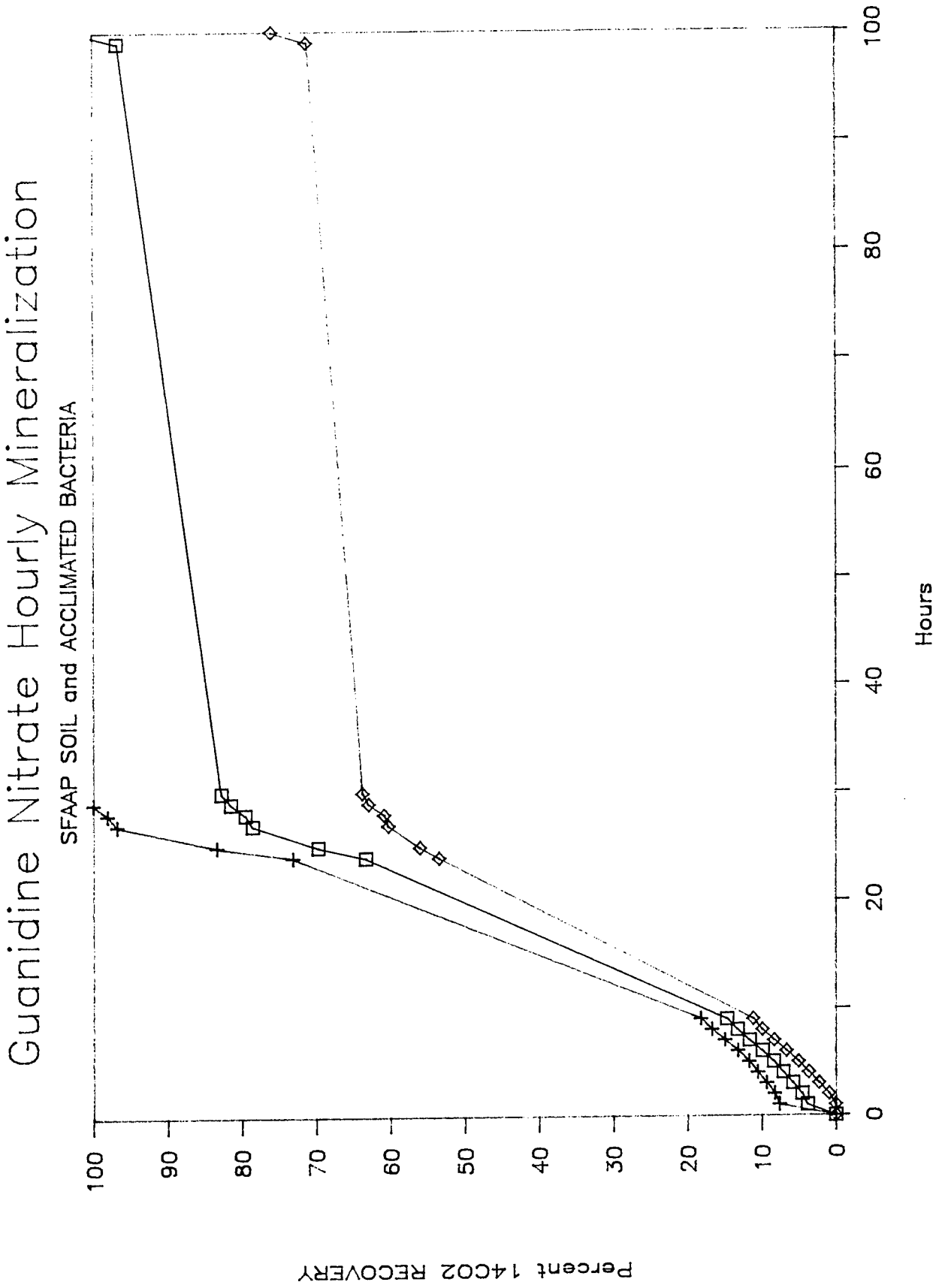

Guanidine Nitrate Hourly Mineralization
SFAAP SOIL and ACCLIMATED BACTERIA
Percent 14CO2 RECOVERY
Hours
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100

Figure 4-18

Figure 4-19

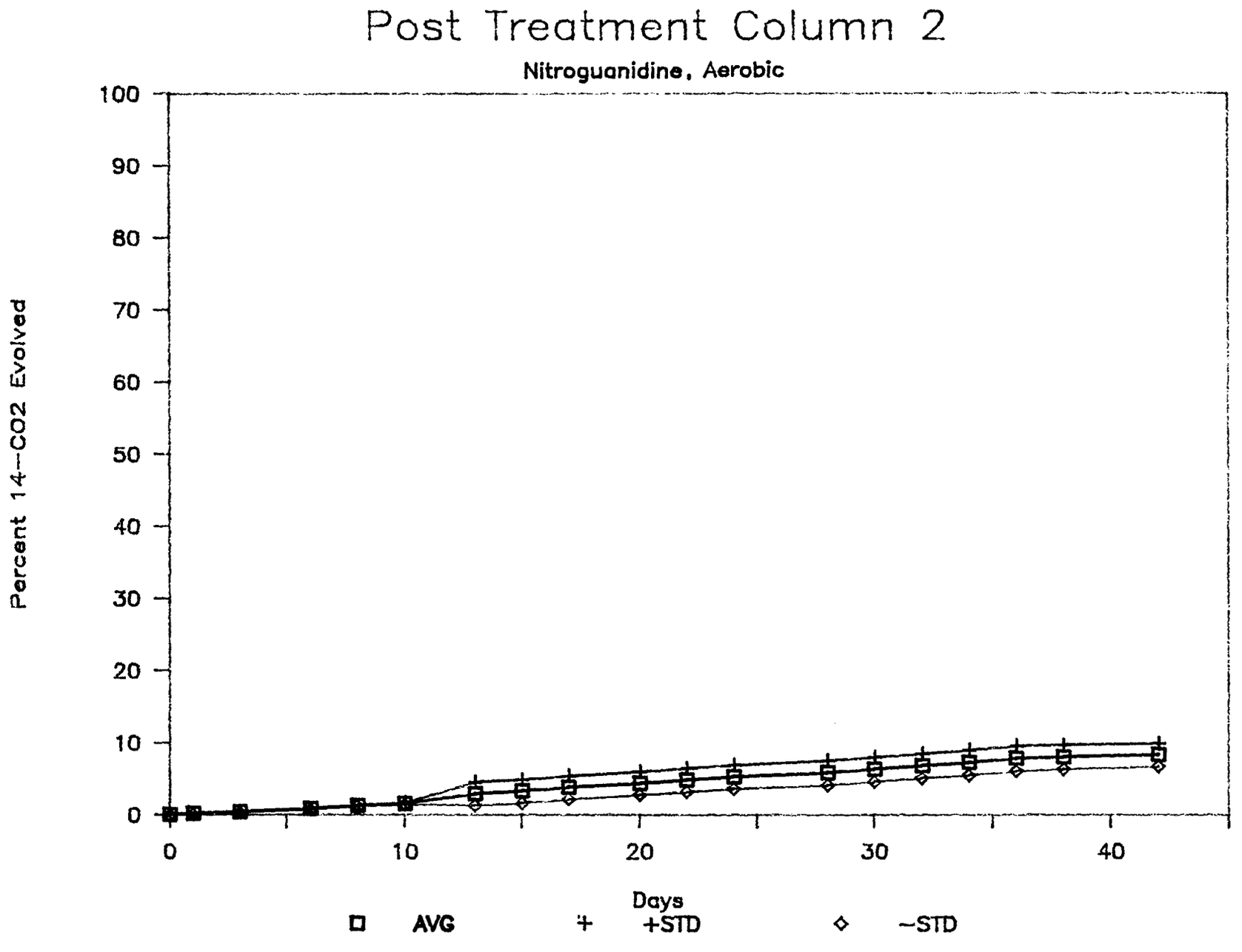
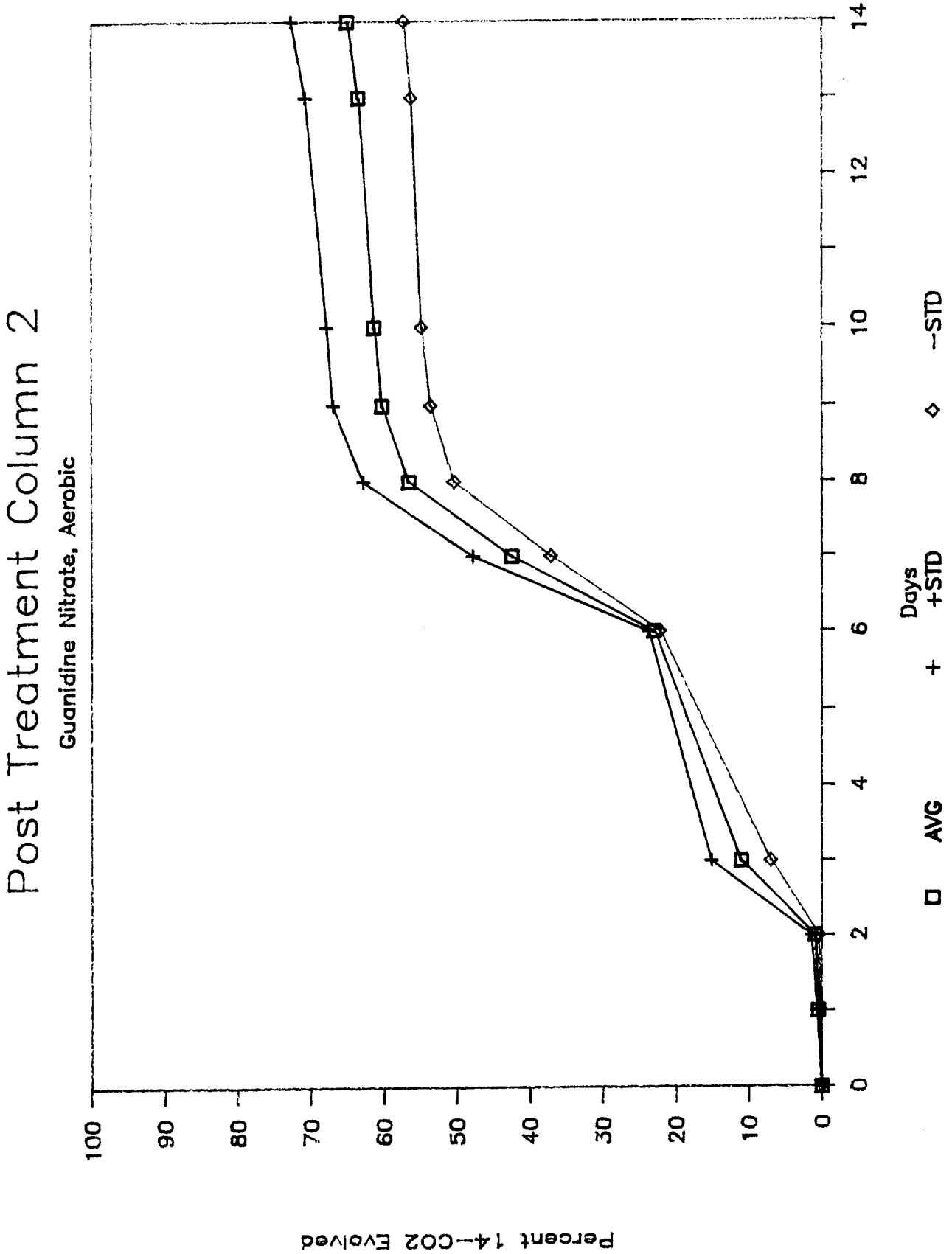

Post Treatment Column 2
Nitroguanidine, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
-STD

Figure 4-20


Post Treatment Column 2
Guanidine Nitrate, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days
AVG
+STD
-STD

Glucose mineralization experiments were conducted as positive controls in post-treatment soil. Results for evolved $^{14}CO_2$ were equal to or slightly higher than those observed in untreated soils. Evolved $^{14}CO_2$ from added $^{14}C$-glucose ranged from 18.5 to 44.4 percent in soil from active columns under aerobic conditions and from 19.0 to 50.0 percent in soil from active columns under anaerobic conditions (Table 4-17). A typical graph of glucose mineralization in post-treatment soil is shown in Figure 4-21. Glucose mineralization curves for all columns are shown in Appendix P.

All three compounds had low mineralization rates in soil from sterile columns 3 and 5 under all test conditions.

Mass balances of $^{14}C$ compounds were determined for representative flasks from mineralization studies as described in Subsection 3.3.3. Isotopic mass balances for the mineralization studies were within ±20 percent of theoretical.

4.4 Enumeration.

4.4.1 Soil column influent and effluent. Enumeration data for soil column influents and effluents are reported in Subsection 4.1.13.

4.4.2 SFAAP soil. Enumerations were done for total number of microorganisms and the number of microorganisms able to degrade GN or NQ with or without supplemental carbon. Pre- and post-treatment soil samples were enumerated.

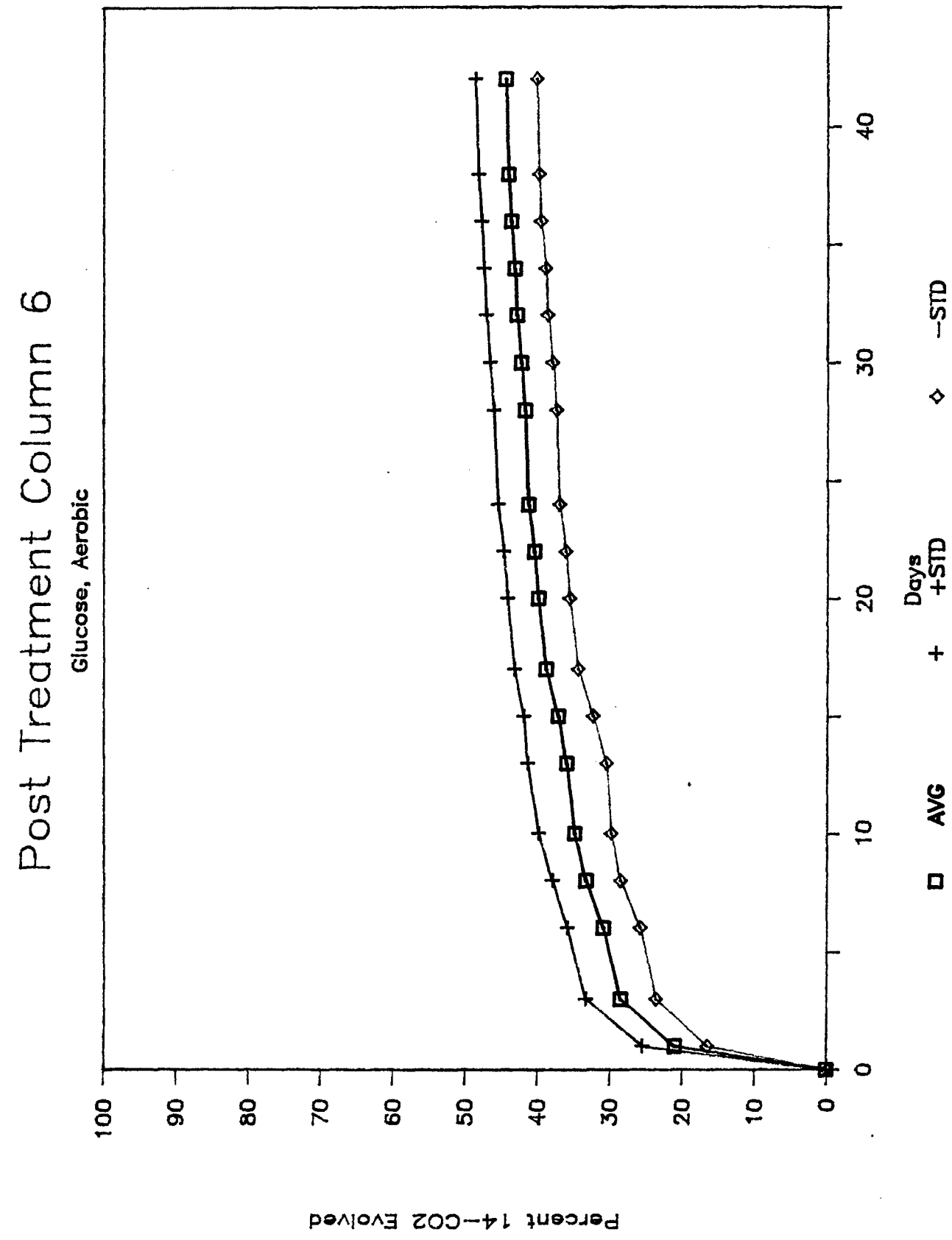
4.4.2.1 Pre-treatment. SFAAP soil, as received, had an average total plate count of $4.8 \times 10^7$ colony forming units per gram (CFU/g). Data from enumerations using total plate count and other media are listed in Table 4-18. All media tested gave similar results.

4.4.2.2 Post-treatment. Post-treatment samples consisted of SFAAP soil taken from continuous flow columns (Subsection 4.1) after completion of the 271-day study. Values for total plate counts were $5.8 \times 10^7$ CFU/g from column 1, $5.9 \times 10^7$ CFU/g from column 2, $2.1 \times 10^7$ CFU/g from column 4 and $1.3 \times 10^7$ CFU/g from column 6. The two sterile columns (3 and 5) had no colony forming units. Enumeration data for all media are listed in Table 4-18. The seven media were used for enumeration gave similar results.

TABLE 4-18. ENUMERATION OF SFAAP SOIL
(CFU/G)

| Media | Pre-treatment | Post-treatment column 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Total Plate Count | $4.8 \times 10^7$ | $5.8 \times 10^7$ | $5.9 \times 10^7$ | 0 | $2.1 \times 10^7$ | 0 | $1.3 \times 10^8$ |
| NQ as Sole Nutrient Source | $3.0 \times 10^6$ | $3.9 \times 10^7$ | $8.1 \times 10^7$ | 0 | $6.5 \times 10^7$ | 0 | $2.0 \times 10^8$ |
| NQ and Dextrose | $4.1 \times 10^6$ | $4.7 \times 10^7$ | $3.5 \times 10^7$ | 0 | $1.1 \times 10^7$ | 0 | $4.5 \times 10^7$ |
| NQ and Whey | $1.5 \times 10^7$ | $2.5 \times 10^7$ | $1.8 \times 10^7$ | 0 | $9.0 \times 10^6$ | 0 | $3.0 \times 10^7$ |
| GN as Sole Nutrient Source | $7.8 \times 10^6$ | $6.1 \times 10^7$ | $5.5 \times 10^7$ | 0 | $3.3 \times 10^7$ | 0 | $3.2 \times 10^8$ |
| GN and Dextrose | $6.4 \times 10^6$ | $4.1 \times 10^7$ | $3.0 \times 10^7$ | 0 | $2.7 \times 10^7$ | 0 | $1.1 \times 10^8$ |
| GN and Whey | $4.9 \times 10^6$ | $1.7 \times 10^7$ | $3.6 \times 10^7$ | 0 | $5.4 \times 10^6$ | 0 | $1.0 \times 10^7$ |

Figure 4-21


Post Treatment Column 6
Glucose, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
-STD

4.4.3 $^{14}$C-MPN. A $^{14}$C-Most-Probable-Number ($^{14}$C-MPN) method for enumeration of microorganisms specifically able to degrade glucose in SFAAP soil was tested. Microbial enumeration of SFAAP soil by $^{14}$C-MPN method proved difficult. Since this method proved ineffective at enumerating total heterotrophic microorganisms, it was not tested for NQ and GN degrading microbes.

4.5 Soil mobility test. The mobility of $^{14}$C-NQ and $^{14}$C-GN in SFAAP soil was tested. A complete data set for the soil mobility test is included in Appendix Q. Approximately 22 percent of the NQ pulse passed through SFAAP soil in 1-4 pore volumes of water (equivalent to 1.5 days). After eight pore volumes had passed through the column, the total effluent volume contained 32 percent of the $^{14}$C-NQ added (Figure 4-22). The rate of passage of $^{14}$C-NQ through the soil peaked at one pore volume and declined dramatically after passage of approximately 3 pore volume.

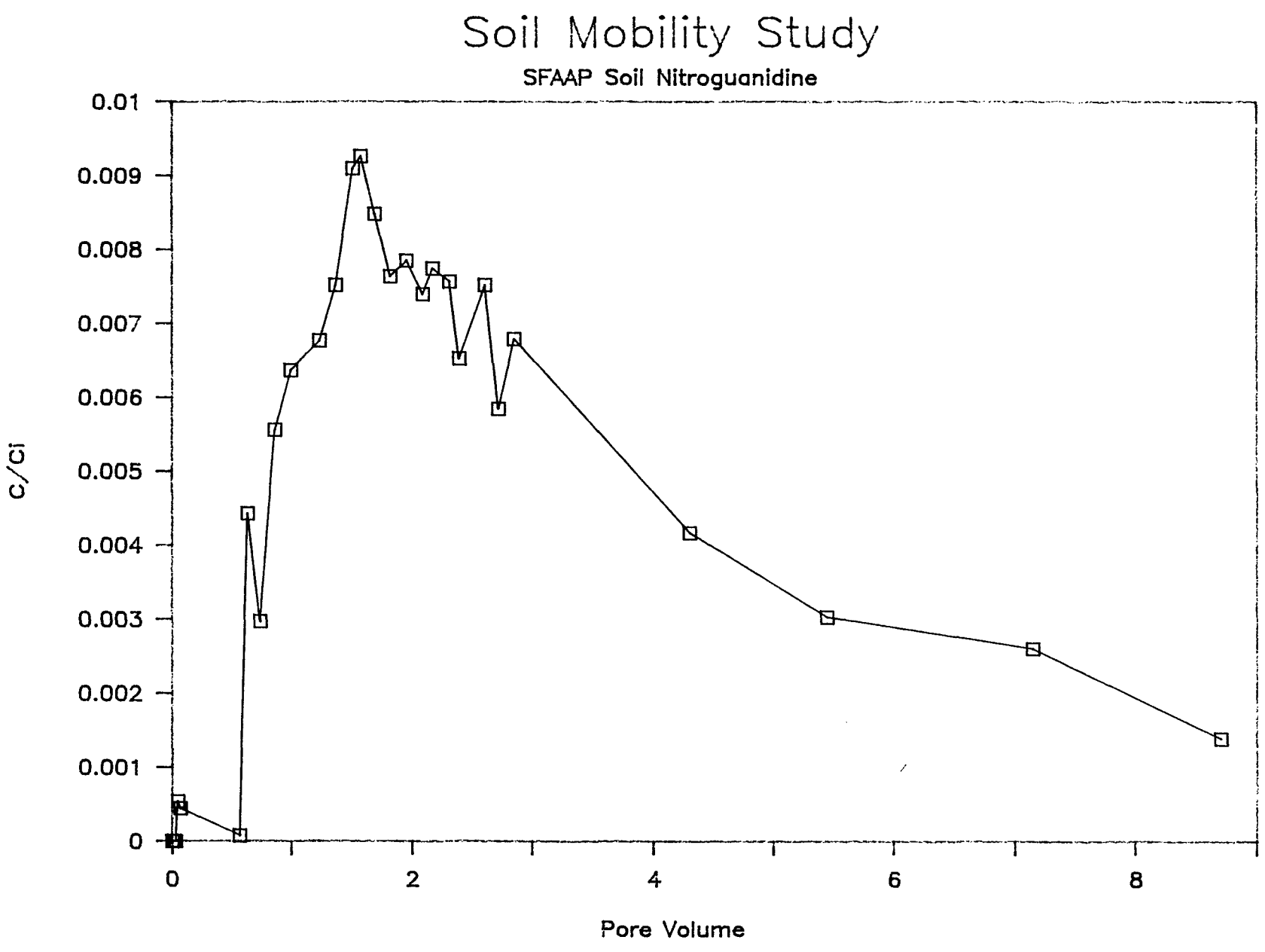
Approximately 19.4 percent of the GN pulse passed through SFAAP soil in 1 to 3 pore volumes of water (equivalent to 1.5-5 days). After 8 pore volumes passed through the column, the total effluent volume contained 36 percent of the $^{14}$C-GN added (Figure 4-23). As with NQ, the rate of passage of GN declined markedly after passage of just over one pore volume (Figure 4-19). The remaining $^{14}$C-NQ and $^{14}$C-GN was retained in the soil. Mass balance calculations indicated 92 percent of theoretical recovery for NQ and 85 percent for GN. The soil within the columns was analyzed for $^{14}$C-NQ or $^{14}$C-GN. The lower section (where the NQ and GN was applied) contained the bulk of the retained $^{14}$C-NQ. The $^{14}$C-GN was more evenly distributed throughout the column. The distributation of the $^{14}$C added to the soil was as follows:

$^{14}$C Distribution In Soil Mobility Columns

| | $^{14}$C-NQ | $^{14}$C-GN |
|---|---|---|
| Top | 6 Percent | 14 Percent |
| Middle | 28 Percent | 25 Percent |
| Lower | 49 Percent | 18 Percent |
| Effluent | 32 Percent | 36 Percent |
| Percent Recovery | 115 Percent | 93 Percent |

4.6 Chemical analysis of soil. Pre- and post-treatment soil was analyzed. Soil from the top and bottom of the soil columns was analyzed. Data are presented in Table 4-4.

Figure 4-22


Soil Mobility Study
SFAAP Soil Nitroguanidine
C/Ci
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
2
4
6
8
Pore Volume

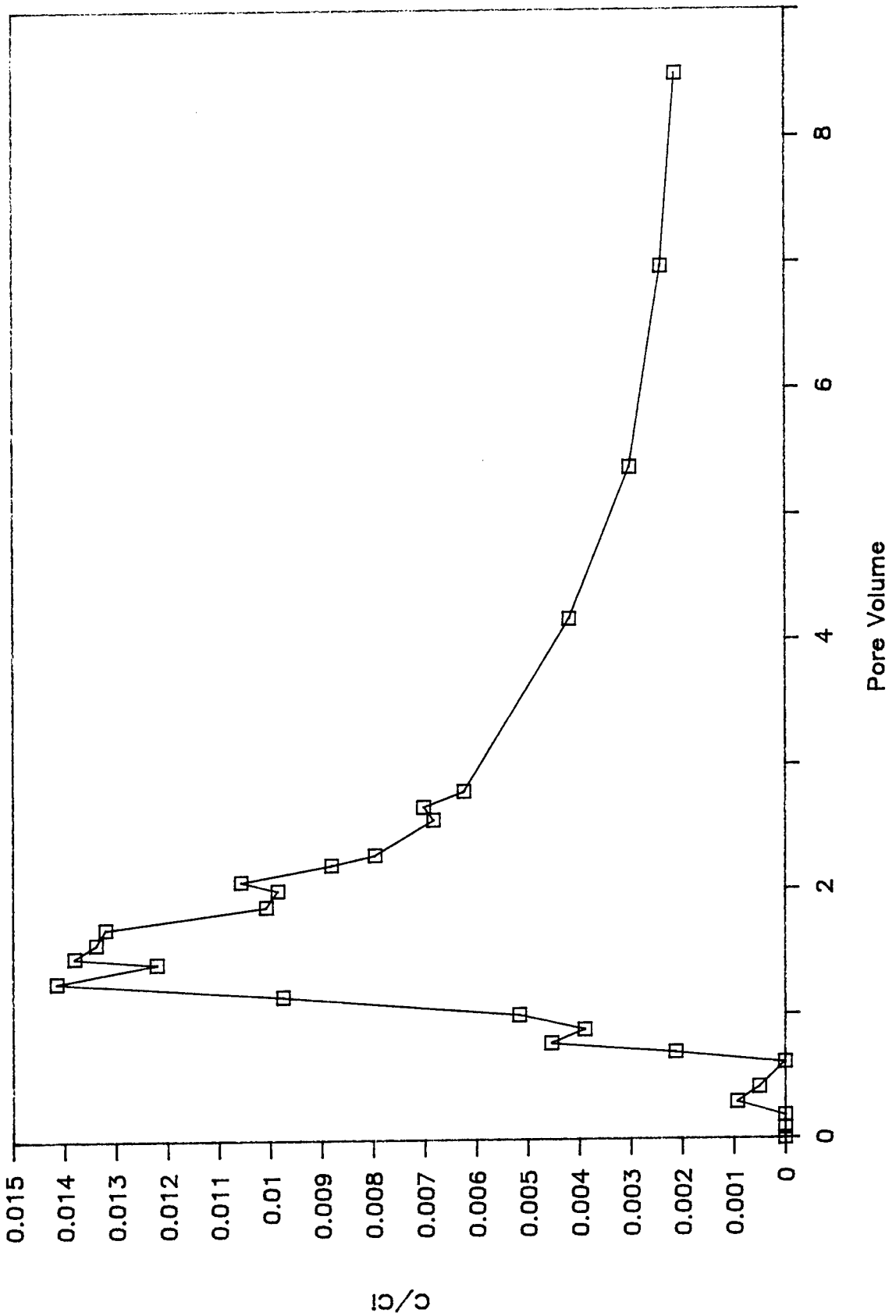

Soil Mobility Study
SFAAP Soil Guanidine Nitrate
C/Ci
Pore Volume
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
2
4
6
8

Figure 4-23

# 5. DISCUSSION

## 5.1 Soil columns.

5.1.1 Nitroguanidine. After 271 days of continuous flow soil column operation, NQ levels were reduced between 30 and 40 percent from the influent to the effluent of three columns. Statistically significant reduction occurred in the active glucose and whey supplemented columns, as well as in the sterile whey supplemented column. Since reduction was observed in both sterile and active columns, the experimental results are inconclusive regarding the influence of microbial activity in NQ removal.

Adsorption of NQ by granular activated carbon has been reported (21). The carbon supplements supplied in the simulated wastewater influents were found to bind to the sterile column soils, as indicated by post-treatment soil TOC levels (Table 4-4). It is possible that NQ sorbed to carbon supplements bound to the column soils. Extracts of the post-treatment column soils do indicate slightly higher NQ levels in the sterile carbon supplemented columns. The columns observed to have the greatest NQ reduction (numbers 4, 5, and 6), also received carbon supplements. Column 3, however, received glucose but did not have a significant reduction in NQ from influent to effluent. The post-treatment soil extract from column 3 did reveal slight adsorption of NQ and organic carbon to column soil. The soil perfusion columns supplemented with carbon did not exhibit significant NQ reduction in the circulated wastewater. Therefore, sorption of NQ to soil-bound carbon, if it occurred, was limited and not uniform.

The NQ degradation data do not duplicate the previously reported 100 percent cometabolic degradation of NQ in soil (7), or the 75 percent cometabolic degradation of NQ to NOQ observed in anaerobic aqueous systems (1). In both of these prior studies, only NQ was supplied rather than simulated wastewater containing guanidine nitrate and inorganic constituents. The previous soil study also did not use SFAAP soil in the soil columns.

The perfusion and continuous flow soil column data indicate that NQ was not significantly transformed, either biologically or nonbiologically. Dr. Richard Bartha, who reviewed the test plan prior to initiation of the study, expressed skepticism regarding the biodegradability of NQ, primarily because of the electron withdrawing property of nitro groups. Biodegradability studies conducted by Polybac Corporation for Hercules revealed that NQ was poorly biotransformed (5). In addition, Dr. W.D. Burrows, who has studied the transformation of NQ and related compounds, was "not surprised" by the low NQ transformation observed (personal communication).

Previous studies have indicated that anaerobiosis facilitates NQ transformation. If aerobic conditions were maintained in the test systems, this could be a possible explanation for the low level of NQ transformation observed. Carbon additives supplement NQ and GN, which have a high nitrogen to carbon ratio. In addition, they facilitate oxygen depleting microbial metabolism. Therefore, it could be suggested that sufficient carbon was supplied to metabolize NQ and GN, but not to create and maintain anaerobic conditions. Glucose, however, was provided at a level that was previously found sufficient for NQ transformation (7). The fact that $^{14}$C-NQ was not significantly mineralized in carbon supplemented flasks specifically established and maintained under anaerobic conditions also refutes this suggestion. In addition, it is highly unlikely that the soil columns remained aerobic, as suggested by the presumptive evidence for sulfate reduction within soil columns.

5.1.2 Nitrosoguanidine. No significant differences were observed in effluent nitrosoguanidine levels between active and sterile columns. Therefore, biodegradation of NQ to NOQ was not conclusively demonstrated. The greatest accumulation of NOQ occurred in the effluents of continuous flow soil columns 3, 5, and 6. These columns had carbon supplements, and (with the exception of the sterile columns) the greatest potential for anaerobic conditions brought about as a result of microbial activity.

No accumulation of NOQ was observed in the soil perfusion columns, where the circulated wastewater was well oxygenated. These results support previous studies where cometabolic degradation of NQ to NOQ was observed in anaerobic aqueous systems (1). However, in the continuous flow soil columns, NOQ was released in the effluent of column 2 (active without carbon supplement), while no accumulation of NOQ occurred in column 4 (active with carbon supplement).

5.1.3 Guanidine. Transformation of guanidine occurred in all continuous flow and soil perfusion columns. The 60-100 percent degradation observed was not linked to either microbial activity or carbon supplement. Consequently, guanidine appears to be relatively susceptible to chemical as well as biological transformation. This is supported by the influent guanidine concentrations, which decreased under sterile conditions during holding time in influent reservoirs. Guanidine was not detected in any of the extracts from post-treatment soil.

5.1.4 Ammonia. Increases in ammonia were observed from influent to effluent within carbon supplemented columns with active microbial populations, but not in either the unsupplemented active column or the two sterile columns. Ammonification of organic wastewater supplements (glucose or whey) is not an adequate explanation for this increase. Glucose contains no nitrogen atoms. Whey, however, is 13 percent protein, and therefore its degradation should result in ammonia production. However, since ammonia is produced in columns supplemented with either glucose or whey, nitroguanidine and guanidine nitrate are likely sources of effluent ammonia. The whey supplemented column did have higher effluent ammonia than the glucose supplemented column, indicating that whey does undergo ammonification, and thereby contributes to effluent ammonia.

The relationship between ammonia production and NQ and/or guanidine nitrate degradation, however, is not straightforward. Ammonia was produced in columns 4 and 6, which also had the highest degradation of NQ. This suggests that ammonia is a product of NQ degradation, which would corroborate a previous NQ biodegradation study (8). That study reported that 85 percent of the NQ nitrogen could be accounted for by ammonia production. Columns 5, 3, and 2 had reductions in both NQ and ammonia levels. Therefore, no direct relationship between NQ or guanidine nitrate degradation and ammonia production can be established based upon data from this study.

Oxidation of ammonia to nitrite-nitrate does not appear to be a factor in reducing ammonia levels in columns 3 and 5 since both are sterile and have reduced ammonia and nitrite-nitrate levels from influent to effluent. Oxidation or assimilation of ammonia may have been a factor in reducing ammonia produced from NQ and GN in column 2.

Denitrification could remove nitrogen from the system as nitrogen gas. However, since such a large percentage of the influent nitrogen was accounted for in the effluent and post-treatment soil, this loss is unlikely to be significant.

5.1.5 Sulfate. The reduction in sulfate in active carbon-supplemented columns suggests that sulfate reduction is occurring. The observation that less sulfate was removed in column 2 indicates that utilizable carbon is the limiting factor for sulfate reduction within the columns. The occurrence of sulfate reduction would confirm the existence of anaerobic conditions within the columns.

Despite this presumptive evidence for sulfate reduction, no characteristic sulfide odor was detected from the columns and no blackened areas were observed when the soil was removed. Column 6, however, did emit a strong odor uncharacteristic of the other columns.

5.1.6 Cyanamide. Cyanamide was detected in all column influents. The most likely source of this cyanamide was chemical transformation of unstable nitrosoguanidine to cyanamide.

The reduction in cyanamide observed from the influent to effluent of all columns indicates that chemical and/or biological transformation was occurring. The cyanamide lost during passage through the columns was not detected on the column soil upon extraction and analysis.

5.1.7 Melamine and cyanoguanidine. Neither of these compounds were detected in the continuous flow soil column influents or effluents, the perfusion column reservoirs, or the post-treatment soils. These compounds may have been formed as transformation products and further decomposed during passage of the wastewater through the soil. However, in the absence of data, this is merely speculation. A previous report (1) indicates that these compounds are not readily degradable.

5.1.8 Total nitrogen balance. Calculation of a total nitrogen balance for the columns confirms that the columns operated without substantial loss of nitrogen. Nitrogen added to the columns was accounted for within ±10 percent for all columns except numbers 2 and 6, where 84 and 87 percent, respectively, of the added nitrogen was accounted for.

Most of the nitrogen added in the influent wastewater passed through the soil columns and was recovered in the effluent. A breakdown of where influent nitrogen was recovered for each column is as follows: column 1, effluent 99 percent, soil 0 percent; column 2, effluent 74 percent, soil 10 percent; column 3, effluent 77 percent, soil 29 percent; column 4, effluent 65 percent, soil 43 percent; column 5, effluent 56 percent, soil 40 percent; column 6, effluent 61 percent, soil 26 percent. See Appendix H for information regarding calculation of the nitrogen balance. Although substantial percentages of total nitrogen were absorbed to soil, extraction of continuous flow soil column soil recovered only small amounts of NQ (Table 4-4).

5.1.9 Total organic carbon. Glucose and whey, added as carbon supplements, were readily metabolized by soil microorganisms. A 50-70 percent reduction in TOC from influent to effluent, as well as reduced TOC levels from pretreatment to post-treatment soil, indicates that the organic carbon added to columns number 4 and 6 was biodegraded. TOC levels were high in the effluents and post-treatment soils of the two sterile columns, indicating that transformation of supplements did not occur in the sterile columns.

5.1.10 pH. Fluctuation in influent pH can partially be explained by the deionized water supply. During the study, the pH of deionized water varied from 5.0 to 7.0.

The effluent pH of columns 1 and 2 was elevated during the study. The effluent of columns 3 and 5 was acidified by the presence of mercuric chloride. The effluent of column 4 could have been made acidic by microbial activity, and the pH of column 6 could have been raised by the presence of ammonia, a likely degradation product of NQ and GN. Since the pH of all columns remained within the metabolic range, microbial activity likely was not affected by the observed variation in pH.

5.1.11 Temperature. The temperature of the columns was maintained between 17 - 26°C during the study. Therefore, temperature may have effected the rate of microbial or chemical transformtion slightly, but not the nature of the transformations. The range of temperature fluctuations was much less than that which would occur at SFAAP. In addition, all columns were uniformly exposed to the gradual temperature changes.

5.1.12 Viability. Columns 3 and 5 were not completely sterilized by the addition of 0.5 percent $HgCl_2$. An increase of $HgCl_2$ to 0.75 percent resulted in sterile effluents.

Influent reservoirs for columns 4 and 6 contained carbon supplement, creating a rich media for microbial growth. Sterile conditions were especially difficult to maintain in column 6 influent, which contained insoluble whey particles. Suspended particles in the reservoir made sterilization difficult. Contaminated reservoirs were replaced immediately after detection of contamination. It is unlikely that the occasional contamination problems affected the study results.

5.1.13 Enumeration. Post-treatment microbial enumerations of column soil were somewhat higher than pretreatment enumerations. Microbial growth in the active columns was likely caused by the constant source of nutrients and carbon. However, large increases or shifts in the total microbial population were not observed. Shifts may have occurred in individual species populations. Enumerations with NQ or GN as sole carbon source were not significantly lower than total plate counts or counts using NQ or GN with carbon supplements. This finding is unusual in light of the level of NQ mineralization observed.

The enumeration data indirectly indicate that NQ and GN were not toxic to microorganisms at the level (0.20 µg/l) used for the enumerations.

5.2 Mineralization. The soil, as received from SFAAP, was physiologically active, as demonstrated by the metabolism of both glucose and an amino acids mixture.

5.2.1 NQ. Mineralization of NQ in SFAAP soil was low under all conditions. The NQ structure, with electron withdrawing nitro groups, appears to be relatively recalcitrant to mineralization based upon these experiments.

Varied concentrations of three carbon supplements, added to induce NQ metabolism, did not enhance NQ mineralization. The addition of nutrients, including phosphorus, or incubation of test flasks under aerobic versus anaerobic conditions, did not increase NQ mineralization. Acclimating microorganisms to NQ by prolonged exposure (271 days) also did not enhance mineralization.

5.2.2 GN. Mineralization of GN in SFAAP soil was rapid and extensive. Guanidine nitrate is an ionic salt which proved to be readily transformed. The rate of GN mineralization at the concentrations tested was not affected by carbon supplement, nutrient addition, or incubation under either aerobic or anaerobic conditions. Acclimation of microoganisms to GN appeared to slightly enhance the mineralization rate.

5.2.3 Evolved $^{14}CO_2$ confirmation. To confirm initial mineralization data for guanidine nitrate, several tests were conducted to determine the volatility of guanidine nitrate. All tests indicate that guanidine nitrate is not a volatile compound, and that collected $^{14}C$ in both NQ and GN experiments is $^{14}CO_2$.

5.3 Soil Mobility. The mobility of NQ and GN in soil is determined by diffusion, adsorption, and migration, as described in Section 2. In the soil mobility study, both NQ and GN were adsorbed to soil containing 1.8 percent TOC. Only 32 and 36 percent of NQ and GN, respectively, passed through the soil columns with the application of seven pore volumes of water. Most of the bound NQ and GN remained in the influent end of the column. In addition, the effluent NQ and GN content decreased markedly after 2 to 3 pore volumes.

When NQ was inadvertently added to continuous flow soil column 1, the initial breakthrough time of NQ occurred within one week. Within two weeks, approximately 80 percent of added NQ had passed through the 1 kg of soil. After five weeks, approximately 90 percent of the NQ had been accounted for in effluent samples. NQ was not found in soil extracts of column 1 at the end of the study. Therefore, NQ did not bind in a persistent manner to the soil of continuous flow column 1. No carbon had been added to column 1 soil, and the soil had received deionized water for over one month previous to NQ addition.

## 6. CONCLUSIONS AND RECOMMENDATIONS

Laboratory experiments indicated that only some components of nitroguanidine wastewater are completely or partially removed during passage through, or incubation in, SFAAP soil. Guanidine nitrate and sulfate are the most readily removed components, although sulfate removal requires supplemental carbon. NQ, the major organic component of NQ wastewater, was poorly degraded under all conditions tested.

Enhanced NQ degradation was not observed in soils exposed to simulated wastewater for 271 days. Microbial adaptation apparently did not occur within this time frame.

Sorption of NQ to soil particles was observed. The capacity of soil for NQ sorption and the stability of the soil/NQ association was not investigated. It is likely, however, that sorption sites would become saturated with time in a land treatment system.

This study indicates that NQ would be poorly removed in a land treatment system and could potentially contaminate groundwater. This possibility would be greatly increased by application of full-strength wastewater. Nitrate also poses a significant environmental problem. Nitrate is negatively charged, and therefore mobile in soil. Nitrate can react with amino compounds to form nitrosamines, which are carcinogenic (22). In addition, nitrate can be reduced in the gastro-intestinal tracts of animals to nitrite, which is toxic. The nitrate content of groundwater is required to be below 10 mg/l at land treatment boundaries.

Hazardous waste regulations may present an impediment to land application in the future. At this time NQ wastes are not listed as hazardous wastes and, therefore, the following does not apply. However, this status could change in the future.

Land treatment of wastes is defined by law as land disposal. The hazardous and solid waste amendments of 1984 prohibit land disposal of hazardous waste after a specific date. Congress has stated that reliance on land disposal should be minimized or eliminated. The only way to avoid this prohibition is to petition on a case-by-case basis to show that there is no migration for as long as the waste remains hazardous. EPA's interpretation of this time is generally considered to be forever (U.S. EPA, personal communication). Consequently, land disposal of hazardous wastes is generally not viewed as a viable treatment technology of the future.

Lagooning of NQ wastewaters that have been pretreated for NQ removal under conditions that encourage denitrification could be a potential solution to the problems presented by high nitrate content. Alternatively, pretreated water may be treatable in a biological treatment system. However, additional testing is required before a proper evaluation of these possibilities can be conducted.

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# APPENDIX A

# ANALYTICAL METHODS

## INTRODUCTION AND OBJECTIVES

Review of several documents[1-4] indicates that nitroguanidine production wastewater may contain, in addition to nitroguanidine and inorganic ions, nitrosoguanidine, cyanoguanidine, guanidine, urea, cyanamide, melamine, and ammeline. Our objective was to develop optimal methodology for each compound individually and then to apply the methodology to wastewaters from Sunflower Army Ammunition Plant (SFAAP).

## MATERIALS AND METHODS

### CHEMICALS

Nitroguanidine (NQ) was purchased (Aldrich Chemical Co.) and purified by recrystallization from water. Nitrosoguanidine (NSQ) was synthesized by zinc dust treatment of NQ according to the published procedure.[5] Cyanoguanidine (CNQ, Eastman Kodak), guanidine hydrochloride (Aldrich), cyanamide (Fisher), melamine (Chemical Service Co.), ammeline (Pfaltz & Bauer), m-phenylenediamine dihydrochloride (Fisher), and sodium pentacyanoammine ferrate (SPF, Fisher) were commercial products used without further purification. The diagnostic test kit used for urea determinations, No. 640, was purchased from Sigma.

### HIGH PRESSURE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSES

A Waters liquid chromatographic system (Waters Associates, Milford, MA) consisted of the following components: two Model 6000A solvent delivery systems, a Model 721 programmable systems controller, a Model 730 data module, a Lamda-max Model 480 LC spectrophotometer, and a Model 710B Waters intelligent sample processor (WISP). A Zorbax $C_8$ reverse phase stainless steel column (25 cm x 4.6 mm ID, particle size 6 µm, DuPont Instruments, Wilmington, DE) was used.

Conditions for NQ, NSQ, and CNQ were as follows: mobile phase, glass-distilled deionized water; flow rate, 0.8 mL/min. Effluent was monitored at 235 nm, 0.05 absorbance units full scale (AUFS). Injection volume was 20 µL. Standard solutions of concentrations 10, 5, 2, 1, and 0.5 mg/L were prepared by dilution of a stock solution freshly prepared each day of analysis.

Conditions for melamine and ammeline were as follows: mobile phase, 28% methanol in 0.005 M octanesulfonic acid adjusted to pH 3 with acetic acid; flow rate 1.5 mL/min. Effluent was monitored at 235 nm, 0.1 AUFS, and injection volume was 200 µL. Standard solutions of concentrations 4, 2, 1, 0.4, and 0.2 mg/L were prepared as above.

Precision and accuracy data for the HPLC analyses are given in Appendix A. Correlation coefficients ($r^2$) were >0.9995.

## ION CHROMATOGRAPHIC ANALYSES

A Dionex Model 16 ion chromatograph, interfaced with a Varian Vista 401 data station and equipped with a Dionex #30831 cation exchange column in conjunction with a cation concentrator pre-column (Dionex #30830), was used to determine guanidine. Eluent was 0.25 mM m-phenylenediamine dihydrochloride in 0.25 mM hydrochloric acid at a flow rate of 2.5 mL/min. The hollow fiber suppressor (Dionex #035352, see Results and Discussion) was regenerated with 0.04 M potassium hydroxide at a flow rate of 2 to 3 mL/min. Samples were injected manually via a 3-mL plastic Luer-Lok syringe into a 100 µL sample loop. The instrument was calibrated by injection of 50, 25, 10, 5, and 1 mg/L standard solutions, prepared from guanidine hydrochloride in water. Response was linear over this range with a typical correlation coefficient of 0.999, and the detection limit (signal to noise ratio 2) was ≤0.5 mg/L. Replicate analyses of samples containing 1, 10, and 40 mg/L are summarized in Table 1.

TABLE 1. PRECISION AND RECOVERY IN GUANIDINE DETERMINATION BY ION CHROMATOGRAPHY

| | Concentration (mg/L) | | | | |
|---|---|---|---|---|---|
| Replicate No. | Low | Medium | High | Low Spike[a] | Medium Spike[a] |
| 1 | 0.99 | 10.1 | 41.0 | 8.79 | 41.9 |
| 2 | 0.90 | 10.4 | 40.8 | 8.89 | 43.2 |
| 3 | 0.91 | 10.9 | 40.7 | 8.84 | 43.2 |
| 4 | 0.96 | 9.8 | 40.4 | 9.00 | 42.8 |
| 5 | 0.97 | 10.4 | 40.8 | 9.08 | 42.9 |
| 6 | 0.94 | 10.2 | 41.0 | 8.87 | 42.5 |
| 7 | 0.94 | 10.3 | 39.7 | 8.89 | 43.0 |
| Mean | 0.94 | 10.3 | 40.6 | 8.91 | 42.8 |
| Std. Deviation | ±0.03 | ±0.34 | ±0.46 | ±0.10 | ±0.46 |
| Rel. Std. Deviation | 3.4% | 3.3% | 1.1% | 1.1% | 1.1% |
| % Recovery[a] | | | | 97% | 99% |

a. Calculations for concentrations of spiked samples and percent recoveries are given in Appendix B.

## SPECTROPHOTOMETRIC ANALYSES

A Beckman 5230 UV/visible spectrophotometer was used for colorimetric determinations of urea and cyanamide. Urea was hydrolyzed by urease and determined by measurement of the absorbance of indophenol at 570 nm. The procedure recommended by Sigma[6] was followed. Cyanamide was determined by measurement of absorbance of the pentacyanoamine ferrate complex at 530 nm.[7,8] Six standard solutions of concentrations over the range 6 to 0.1 mg/L were freshly prepared each day of analysis by dilution of a stock solution of 0.1 M

cyanamide (~.105 g/L). The stock solution was prepared once a week and kept refrigerated. SPF solution (0.02 M) was freshly prepared daily. Three 2-mL replicates of each standard solution were added to test tubes containing 0.2 M pH 10.5 sodium carbonate buffer[7] (1 mL) and SPF solution (1 mL). The mixtures were shaken thoroughly and allowed to stand 45 min before absorbance readings at 530 nm were taken. Reagent blanks were subtracted from the readings. Precision and recovery data are listed in Appendix C; correlation coefficients were 0.9999.

THIN LAYER CHROMATOGRAPHIC (TLC) ANALYSES

Cellulose plates were used and were developed in the following systems: 3N $NH_4OH$ methanol (60:75, system 1), n-butanol/ethanol/water (4:1:1, system 2), and 1-propanol/conc $NH_4OH$/water (8:1:1, system 3). Samples were applied to the plates from methanol solutions, except in the case of ammeline, which was very sparingly soluble in water and hydroxylic solvents and was applied from 5N formic acid solution. In most cases optimum visualization of the spots was achieved by dipping in 3N $NH_4OH$/0.1N $AgNO_3$ (1:1) followed by air-drying and heating 10 min at 100°. CNQ and cyanamide were detected by ferricyanide/nitroprusside spray reagent[9] (FCNP) and urea by p-dimethylamino-benzaldehyde/1N HCl[9] (DAB) spray.

## RESULTS AND DISCUSSION

HPLC proved to be the method of choice for all ultraviolet-absorbing compounds, which include NQ, NSQ, CNQ, melamine, and ammeline. Wastewater samples could conveniently be injected onto the column without extraction or pretreatment. Detection limits and retention times are summarized in Table 2. Sensitivity for NQ at 235 nm was found comparable to that reported previously at 263 nm,[3,10] while sensitivity for NSQ at 235 nm was tenfold greater. The use of water as mobile phase afforded better resolution and more efficient yet rapid separation of the substituted guanidines.

TABLE 2. HPLC ANALYSES OF POSSIBLE NITROGUANIDINE WASTEWATER CONSTITUENTS

| Compound | Low Standard (mg/L) | Injection Volume (µL) | Detection Limit[a] (µg/L) | Retention Time (min) |
|---|---|---|---|---|
| Nitroguanidine | 0.50 | 20 | 100 | 6.0 |
| Nitrosoguanidine | 0.50 | 20 | 42 | 4.6 |
| Cyanoguanidine | 0.51 | 20 | 170 | 5.4 |
| Melamine | 0.21 | 200 | 28 | 10.1 |
| Ammeline | 0.20 | 200 | 21 | 9.2 |

a. Signal to noise ratio 2.

Typical injections of standards for NQ, NSQ, and CNQ, and for ammeline and melamine are depicted in Figures 1 and 2, respectively. Figure 3 illustrates a typical HPLC analysis of NQ process wastewater in which ammeline at 0.38 mg/L and melamine at 0.23 mg/L were detected in tank 105 before treatment at SFAAP. After treatment, 0.089 mg/L ammeline remained, and melamine was below detection limit. For analyses of these and other SFAAP wastewater samples for other constituents, see Methods Application section.

Guanidine, not amenable to HPLC detection, was optimally determined conductimetrically as the cation by ion chromatography. The method necessitates utilization of a suppressor to reduce the background conductivity of the eluent which in turn enhances the conductivity signal of the analyte. During initial attempts using a suppressor resin, successive sample injections resulted in increasingly longer retention times. This problem, attributed to possible interaction of guanidinium ion or nitroguanidine with the suppressor resin, was eliminated by replacing the suppressor resin with a fiber suppressor. With this system, anions are exchanged through a membrane wall, thus minimizing any undesirable interactions.

Under the previously described conditions, the retention time of guanidinium ion is 5.1 min. Common monovalent cations, e.g., $Na^+$, $K^+$, and $NH_4^+$, have shorter retention times (1.6 to 2.0 min) and do not interfere. Divalent cations, e.g., $Ca^{++}$ and $Mg^{++}$, elute in excess of 30 min. In summary, the method appears to be highly reproducible, with few interferences and adequate sensitivity. It should be noted, however, that during development of the method the cation column began to turn pink. This was attributed to slow polymerization of m-phenylenediamine and attachment of the polymer to the resin. There was no immediate effect on the separations, and it was found that polymerization was minimal if air was excluded from eluent reservoirs and columns were covered with aluminum foil to exclude light. Under these conditions, cation columns should last 6 months or longer.

Cyanamide also could not be analyzed by HPLC, but was determined spectrophotometrically by complexation with pentacyanoammine ferrate reagent.[7] The method is specific for cyanamide and was not subject to interferences by other organic constituents of NQ production wastewater. Detection limits were below 0.1 mg/L unless high concentrations of inorganic salts were present.

TLC separations of the expected NQ wastewater constituents were also investigated, and optimum parameters are summarized in Table 3. Several disadvantages are readily apparent. Detection limits are frequently greater by several powers of ten relative to HPLC, and the spots, visualized by chromogenic spray or dip reagents (see Table 3), cannot be readily quantitated. Furthermore, interferences from dissolved inorganic salts in wastewaters preclude direct application of aqueous solutions to the plates, and the organic constituents are generally too polar for efficient extraction by organic solvents.

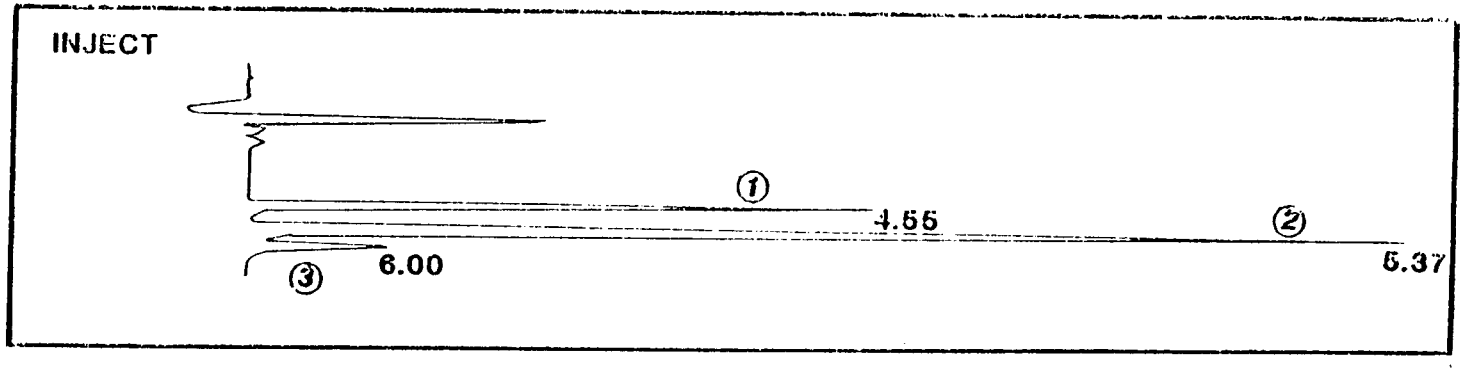


Figure 1. HPLC standards: nitrosoguanidine (1, 1.72 mg/L), cyanoguanidine (2, 5.09 mg/L), nitroguanidine (3, 0.33 mg/L).

INJECT
10.03
INJECT
(a)
(b)
TIME (min)

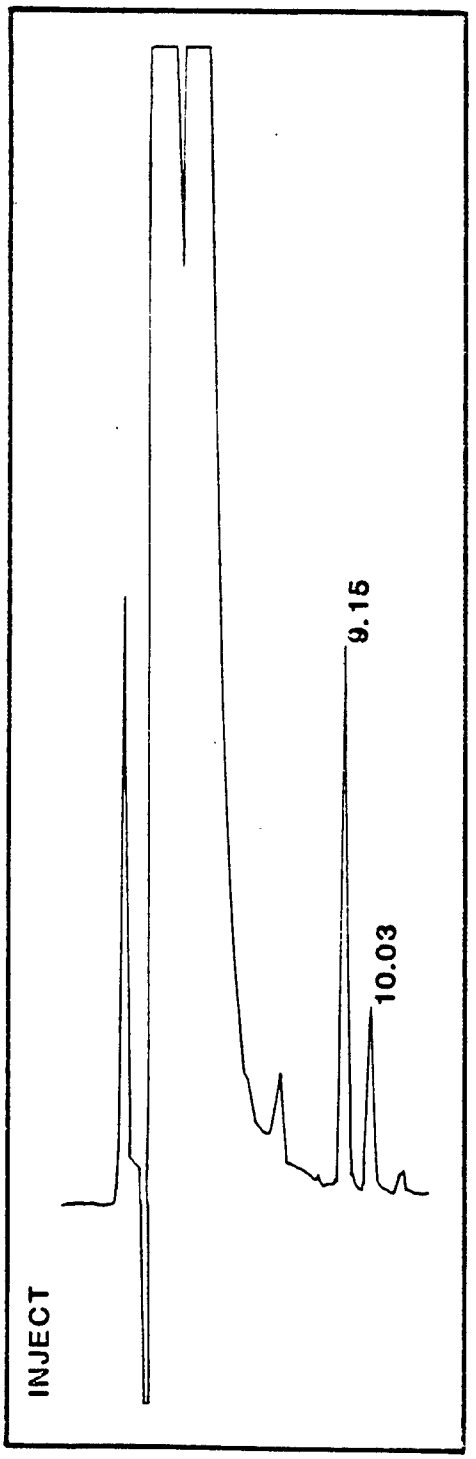


Figure 3. HPLC analysis of tank 105, Sunflower Army Ammunition Plant, before treatment.

TABLE 3. TLC PARAMETERS FOR POSSIBLE NITROGUANIDINE WASTEWATER CONSTITUENTS

| Compound | Optimum Solvent System | Chromogenic Reagent | Color | $R_F$ | Detection (µg) |
|---|---|---|---|---|---|
| Guanidine | 1 | $AgNO_3/NH_4OH$ | Brown/Brown BG | 0.8 | 2 |
| Cyanoguanidine | 2 | FCNP | Pink-purple | 0.45 | 1 |
| Melamine | 2 | $AgNO_3/NH_4OH$ | White/Brown BG | 0.25 | 0.5 |
| Ammeline | 2 | $AgNO_3/NH_4OH$ | Brown/Brown BG | 0.45 | 5 |
| Cyanamide | 2 | FCNP | Pink-purple | 0.8 | 0.2 |
| Urea | 3 | DAB | Yellow | 0.6 | 1 |

## METHODS APPLICATION

While methods development was at an early stage (November 1982), water samples were taken from certain SFAAP locations for analysis. Because the samples were stored (under refrigeration) for at least several months prior to analysis of trace organics, those results (Table 4) may be considered as only indicative of the original content. Table 5 summarizes recent analyses (October 1983) of wastewater from Tank 105, before and after treatment with lime/steam. The sample after treatment was, at our request, neutralized with HCl to prevent possible further reaction on standing. Because dimerization of cyanamide to CNQ is rapid at pH >7, and very little of the latter was detected, cyanamide was not sought.

TABLE 4. ANALYSES OF SFAAP WATER[a]

| Analyte (mg/L) | Location (pH) | | | |
|---|---|---|---|---|
| | Trailer (9.6) | NQ SE Sump (11.3) | Basin 123 (7.3) | Wet NQ Sump (8.8) |
| NQ | 2 | 327 | 0.3 | 915 |
| CNQ | ND | ND | 1.51 | <0.17 |
| NSQ | ND | ND | <0.042 | 0.43 |
| Ammeline | ND | ND | <0.021 | <0.021 |
| Melamine | ND | ND | 0.084 | 0.060 |
| Guanidine | 85 | 85 | 63 | ND |
| TKN | 700 | 1,150 | 125 | 330 |
| $NH_3$-N | 140 | 235 | 75 | ND |
| $Cl^-$ | 30 | 30 | 20 | 180 |
| $NO_2$- | 360 | 745 | 7 | 5 |
| $NO_3$- | 14 | 13 | 845 | 110 |
| $SO_4^=$ | 190 | 215 | 59 | 1,690 |

a. ND - not determined.

TABLE 5. ANALYSIS OF WASTEWATER FROM SFAAP TANK 105 (mg/L)

| Analyte | Before Treatment (pH 8.2) | After Treatment[a] (pH 6.9) |
|---|---|---|
| NQ | 2849 | 0.54 |
| CNQ | <0.17 | <0.17 |
| NSQ | <0.042 | <0.042 |
| Ammeline | 0.377 | 0.089 |
| Melamine | 0.230 | <0.028 |
| Guanidine[b] | - | 10.8[c] |
| Urea | <15 | 1,240[c] |
| TKN | 659 | 985 |
| $NH_3$-N | 5.5 | 40.5 |
| $Cl^-$ | 130 | >400[d] |
| $NO_2^-$ | 20 | 840[c] |
| $NO_3^-$ | 1.8 | 1.6 |
| $SO_4^=$ | 98 | 80 |

a. Neutralized, not corrected for dilution.
b. Not possible to determine in presence of very large excess of NQ.
c. Formed from NQ by treatment.
d. From HCl added to neutralize sample.

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APPENDIX A-1

PRECISION AND ACCURACY OF HPLC ANALYSES OF
NQ, NSQ, CNQ, MELAMINE, AND AMMELINE

PRECISION

Precision of the method was determined by injecting a sample four times on three separate days. Mean, standard deviation, and relative standard deviation were calculated for a low and high concentration.

1. Nitroguanidine

| Day | Date | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 7 July 83 | 0.220 | 0.010 | 4.54 |
| 2 | 13 July 83 | 0.220 | 0.004 | 1.82 |
| 3 | 14 July 83 | 0.220 | 0.010 | 4.54 |
| | Overall | 0.220 | 0.008 | 3.63 |
| | | High Concentration | | |
| 1 | 7 July 83 | 5.05 | 0.03 | 0.59 |
| 2 | 13 July 83 | 5.03 | 0.02 | 0.40 |
| 3 | 14 July 83 | 5.03 | 0.03 | 0.60 |
| | Overall | 5.04 | 0.03 | 0.53 |

2. Nitrosoguanidine

| Day | Date | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 1 Aug 83 | 0.50 | 0.01 | 2.00 |
| 2 | 2 Aug 83 | 0.50 | 0.02 | 4.00 |
| 3 | 4 Aug 83 | 0.49 | 0.01 | 2.04 |
| | Overall | 0.50 | 0.01 | 2.68 |
| | | High Concentration | | |
| 1 | 1 Aug 83 | 10.22 | 0.06 | 0.59 |
| 2 | 2 Aug 83 | 10.33 | 0.07 | 0.68 |
| 3 | 4 Aug 83 | 10.02 | 0.06 | 0.60 |
| | Overall | 10.19 | 0.06 | 0.62 |

3. Cyanoguanidine

| Day | Date | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 25 July 83 | 0.49 | 0.01 | 2.04 |
| 2 | 26 July 83 | 0.48 | 0.01 | 2.08 |
| 3 | 27 July 83 | 0.49 | 0.02 | 4.08 |
| | Overall | 0.49 | 0.01 | 2.73 |
| | | High Concentration | | |
| 1 | 25 July 83 | 10.23 | 0.08 | 0.78 |
| 2 | 26 July 83 | 10.15 | 0.02 | 0.20 |
| 3 | 27 July 83 | 10.38 | 0.02 | 0.19 |
| | Overall | 10.25 | 0.04 | 0.39 |

4. Melamine

| Day | Date | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 24 May 83 | 0.21 | 0.01 | 4.76 |
| 2 | 25 May 83 | 0.21 | 0.01 | 4.76 |
| 3 | 26 May 83 | 0.21 | 0.01 | 4.76 |
| | Overall | 0.21 | 0.01 | 4.76 |
| | | High Concentration | | |
| 1 | 24 May 83 | 2.10 | 0.01 | 0.48 |
| 2 | 25 May 83 | 2.10 | 0.01 | 0.48 |
| 3 | 26 May 83 | 2.09 | 0.01 | 0.48 |
| | Overall | 2.10 | 0.01 | 0.48 |

5. Ammeline

| Day | Date | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 31 May 83 | 0.19 | 0.01 | 5.26 |
| 2 | 01 June 83 | 0.18 | 0.01 | 5.56 |
| 3 | 02 June 83 | 0.19 | 0.01 | 5.26 |
| | Overall | 0.19 | 0.01 | 5.36 |
| | | High Concentration | | |
| 1 | 31 May 83 | 2.06 | 0.01 | 0.40 |
| 2 | 01 June 83 | 2.04 | 0.02 | 0.98 |
| 3 | 02 June 83 | 2.03 | 0.02 | 0.99 |
| | Overall | 2.04 | 0.02 | 0.82 |

## ACCURACY

Accuracy is better defined as percent recovery. This is determined by taking an aliquot of a sample of low concentration and adding a spike to double the concentration. The aliquot is then analyzed four times to obtain a mean, standard deviation, relative standard deviation and percent recovery. This is repeated for a sample of high concentration.

1. Nitroguanidine

| Day | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 1.53 | 0.01 | 0.65 | 104.79 |
| 2 | 1.51 | 0.01 | 0.66 | 100.00 |
| 3 | 1.54 | 0.01 | 0.65 | 96.86 |
| | | | | 100.55 |
| | | High Level | | |
| 1 | 7.46 | 0.01 | 0.13 | 101.08 |
| 2 | 7.34 | 0.02 | 0.27 | 100.96 |
| 3 | 7.34 | 0.05 | 0.68 | 100.96 |
| | | | | 101.00 |

2. Nitrosoguanidine

| Day | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 1.61 | 0.01 | 0.62 | 101.90 |
| 2 | 1.70 | 0.05 | 2.94 | 98.27 |
| 3 | 1.54 | 0.05 | 4.55 | 100.65 |
| | | | | 100.27 |
| | | High Level | | |
| 1 | 7.44 | 0.04 | 0.54 | 100.54 |
| 2 | 7.45 | 0.04 | 0.54 | 99.33 |
| 3 | 7.18 | 0.05 | 0.70 | 98.49 |
| | | | | 99.45 |

3. Cyanoguanidine

| Day | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 1.51 | 0.01 | 0.66 | 100.67 |
| 2 | 1.50 | 0.02 | 1.33 | 100.00 |
| 3 | 1.65 | 0.02 | 1.21 | 102.48 |
| | | | | 101.05 |
| | | High Level | | |
| 1 | 7.55 | 0.02 | 0.26 | 100.94 |
| 2 | 7.39 | 0.03 | 0.41 | 100.14 |
| 3 | 7.66 | 0.06 | 0.78 | 102.00 |
| | | | | 101.03 |

4. Melamine

| Day | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 0.62 | 0.01 | 1.61 | 101.64 |
| 2 | 0.61 | 0.01 | 1.64 | 100.00 |
| 3 | 0.61 | 0.01 | 1.64 | 100.00 |
| | | | | 100.55 |
| | | Medium Level | | |
| 1 | 2.97 | 0.01 | 0.34 | 100.34 |
| 2 | 2.96 | 0.01 | 0.34 | 100.00 |
| 3 | 2.93 | 0.01 | 0.34 | 98.99 |
| | | | | 95.03 |

5. Ammeline

| Day | Mean (mg/L) | Standard Deviation (±) | Relative Standard Deviation (%) | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 0.59 | 0.01 | 1.69 | 101.72 |
| 2 | 0.59 | 0.01 | 1.69 | 101.72 |
| 3 | 0.59 | 0.01 | 3.34 | 101.72 |
| | | | | 101.72 |
| | | Medium Level | | |
| 1 | 2.88 | 0.01 | 0.35 | 100.77 |
| 2 | 2.87 | 0.02 | 0.70 | 101.41 |
| 3 | 2.88 | 0.02 | 0.69 | 101.77 |
| | | | | 101.65 |

## APPENDIX A-2

### CONCENTRATIONS OF SPIKED GUANIDINE SAMPLES

1. Low spike:

   1 mL of 0.94 mg/L + 10 mL of 10 mg/L = 9.18 mg/L

2. Medium spike:

   2 mL of 10.3 mg/L + 10 mL of 50 mg/L = 43.4 mg/L

### PERCENT RECOVERIES OF SPIKED GUANIDINE SAMPLES

1. Low spike:

   8.91/9.18 x 100 = 97%

2. Medium spike:

   42.8/43.4 x 100 = 99%

APPENDIX A-3

## PRECISION AND RECOVERY IN SPECTROPHOTOMETRIC ANALYSIS OF CYANAMIDE

### PRECISION

Precision of the method was determined by analysis of three replicates each of low and high concentration samples on three separate days.

| Day | Date | Mean (mg/L) | Standard Deviation ± | Relative Standard Deviation % |
|---|---|---|---|---|
| | | Low Concentration | | |
| 1 | 16 Jan 84 | 0.332 | 0.000 | 0.00 |
| 2 | 19 Jan 84 | 0.330 | 0.014 | 4.32 |
| 3 | 20 Jan 84 | 0.330 | 0.000 | 0.00 |
| | Overall | 0.331 | 0.005 | 1.44 |
| | | High Concentration | | |
| 1 | 16 Jan 84 | 5.22 | 0.027 | 0.52 |
| 2 | 19 Jan 84 | 5.27 | 0.024 | 0.45 |
| 3 | 20 Jan 84 | 5.24 | 0.016 | 0.31 |
| | Overall | 5.25 | 0.022 | 0.43 |

### RECOVERY

Recovery was determined by analysis of three replicates each of low and high concentration samples spiked to double the concentrations.

| Day | Mean (mg/L) | Standard Deviation ± | Relative Standard Deviation % | % Accuracy |
|---|---|---|---|---|
| | | Low Level | | |
| 1 | 0.361 | 0.000 | 0.00 | 103.38 |
| 2 | 0.350 | 0.014 | 3.96 | 100.53 |
| 3 | 0.351 | 0.016 | 4.68 | 100.57 |
| | | | | 101.49 |
| | | High Level | | |
| 1 | 2.02 | 0.041 | 2.00 | 102.32 |
| 2 | 2.01 | 0.027 | 1.36 | 101.96 |
| 3 | 2.03 | 0.027 | 1.31 | 103.53 |
| | | | | 102.60 |

Reference

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# Spectrophotometric Determination of Cyanamide

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DONALD A. BUYSKE and VINCENT DOWNING

*Experimental Therapeutics Research, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.*

▶ A spectrophotometric method for the quantitative determination of cyanamide in complex mixtures has been developed. This procedure measures the absorbance at 530 mμ of the red complex that is formed when cyanamide and pentacyanoammineferrate interact at pH 10.5 in carbonate buffer. The lower limit of sensitivity is about 1 μg. Recoveries from blood, urine, and soil are 93% or better. Contaminants or additives often found in cyanamide preparations do not interfere with the determination.

PRELIMINARY to studies on the pharmacology of calcium cyanamide and on the mechanism of action of this compound in the treatment of alcoholism, a simple, sensitive, and specific method of determination was desirable. In the diverse uses of calcium cyanamide as a chemical intermediate as well as in its application in agriculture as a fertilizer, the methods for determination most widely used involved the formation of an insoluble silver salt complex, followed by a total nitrogen determination (*1*) or a back-titration of excess silver ion with potassium thiocyanate (*5, 8, 10*). Recently a spot test procedure (*7*) and an efficient paper chromatographic method (*9*) for the separation and qualitative detection of microgram quantities of cyanamide have been described. Since none of these procedures had the specificity or sensitivity required for the quantitative determination of the compound in complex mixtures, an alternative method was sought.

A promising lead was suggested in the work of Buchanan and Barsky (*4*), who studied the fleeting red color which was occasionally observed when iron-containing ores were treated with a solution of crude sodium cyanide. They established that this color was due to an interaction of calcium cyanamide, which was present as an impurity, with a complex of ferrocyanide ion.

The work described herein is an adaptation of the observations reported by these earlier workers, and their extension to a simple spectrophotometric method of the determination for cyanamide in various mixtures.

## EXPERIMENTAL

**Apparatus.** A Beckman Model B spectrophotometer with Corex cells of 1.002-cm. light path was used for the cyanamide determinations. All measurements of pH were done with a Leeds & Northrup Model 7664 pH meter.

**Reagents.** A standard solution of cyanamide was prepared by dissolving 20 mg. of powdered reagent grade calcium cyanamide in 20 ml. of 0.1*N* hydrochloric acid. Sufficient 1.0*N* sodium hydroxide was then added to increase the pH to 7 and water was added to yield a total solution volume of 100 ml. This solution contained 200 μg. of calcium cyanamide or 105 μg. of cyanamide per ml.

A buffer solution was prepared by the slow addition of concentrated hydrochloric acid to a 0.2*M* solution of sodium carbonate until the pH was lowered to 10.5.

An aqueous solution of 0.2*M* trisodium pentacyanoammineferrate, $Na_3[Fe(CN)_5NH_3]$ (K and K Laboratories, Inc., Long Island City, N. Y.), was prepared.

**Preparation of Standard Curve.** The standard calcium cyanamide solution was added in 0.1-, 0.2-, 0.3-, 0.4-, and 0.5-ml. amounts to test tubes containing 2.0 ml. of buffer and 0.2 ml. of 0.2*M* $Na_3[Fe(CN)_5NH_3]$. Water was added to bring the total volume to 3.5 ml. A tube containing 2 ml. of buffer, 0.2 ml. of $Na_3[Fe(CN)_5NH_3]$, and 1.3 ml. of water but no cyanamide provided the reagent blank solution. This reagent solution had an absorbance of less than 0.05 when read at 530 mμ against distilled water.

After the contents had been mixed, each tube was allowed to stand for 45 minutes under the normal conditions of laboratory light and temperature and its absorbance at 530 mμ determined against the reagent black. A plot of absorbance *vs.* concentration yielded a straight line which intersected the origin. This graph served as a standard curve for the determination of cyanamide in the concentration range of 0 to 52.5 μg. per sample tube.

**Determination in Soil.** One hundred milliliters of 0.1*N* hydrochloric acid was added to 30 grams of soil, the mixture was stirred vigorously for 30 minutes and filtered, and sodium hydroxide was added to a pH of 7. This solution was then assayed directly and the concentration of cyanamide found by a comparison with the standard curve.

**Determination in Blood.** Whole dog blood was collected in a glass-stoppered tube containing sodium fluoride as anticoagulant. An equal volume of a solution of 10% trichloroacetic acid in water was added to the blood and the tube shaken vigorously. After standing for 20 minutes, the sample was centrifuged at 2100 r.p.m. for 10 minutes.

The supernatant liquids were filtered through Whatman No. 2 filter paper and the filtrate was adjusted to pH 7 before analysis.

**Determination in Urine.** Urine was assayed directly, provided it was not too heavily pigmented. An alternative procedure was to adjust the urine to pH 2 with concentrated hydrochloric acid and to extract with 10 volumes of ethyl acetate which previously had been equilibrated with 0.01*N* hydrochloric acid. The ethyl acetate was removed by in vacuo distillation on a rotary evaporator, and the residue in the flask dissolved in the buffer solution for direct analysis.

## RESULTS

The absorption spectra for the red complex of the pentacyanoammineferrate with a solution of calcium cyanamide are shown in Figure 1.

Because cyanamide is relatively unstable and not readily available, the calcium complex was used as a source for free cyanamide. This was justified, because solutions of freshly prepared cyanamide containing no calcium and a solution of calcium cyanamide produced identical absorption spectra when each reacted with the pentacyanoammineferrate reagent. When calculated on the basis of cyanamide equivalents, an absorptivity of 75 was obtained for both these preparations.

A study of the conditions for the reaction (Table I) resulted in the selection of pH 10.5, a reagent volume of 0.2 ml., and a reaction time of 45 minutes as an optimum combination for the maximum development of color. For each determination in Table I, 40 μg. of calcium cyanamide was assayed in a buffer volume of 2.0 ml. and a total volume of all ingredients of 3.5 ml. per tube. When a 0.2-ml. cell with a light path of 1.0 cm. was used and the volumes of the buffer, reagent, and sample were appropriately reduced, the lower limit of the sensitivity of the method was less than 1 μg. per determination.

Compounds that are associated with cyanamide as a result of degradation and polymerization or are present as

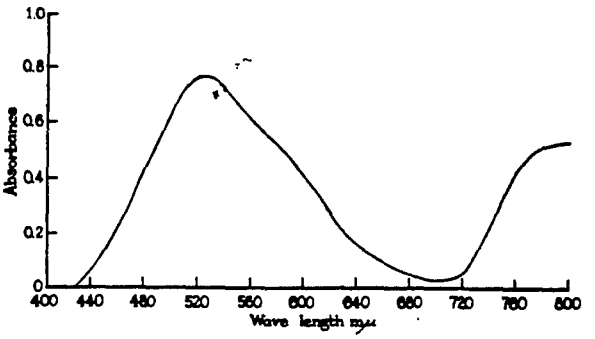



**Figure 1. Absorption spectra of the product of the reaction of cyanamide with pentacyanoammineferrate in alkaline aqueous solution**

contaminants or additives in special preparations include urea, ammonia, dicyandiamide, melamine, guanidine, cyanide, and citric acid. Table II shows the absorbance at 530 mμ when solutions containing these compounds with and without calcium cyanamide are determined by the method described. The compounds tested were present at a concentration five times higher than that of calcium cyanamide or almost ten times higher than the concentration of cyanamide equivalents. The detectable absorbance noted for dicyandiamide may be due to a small impurity of cyanamide. When present in concentrations equal to that of cyanamide, none of the compounds in Table II caused any inhibition or increase in the development of the red complex.

When solutions of 5 to 40 μg. of calcium cyanamide per ml. of whole heparenized blood or urine were prepared and determined by the method described in the procedure section, recoveries varied from 80 to 90% and from 78 to 82%, respectively. If a second extraction of urine with 10 volumes of fresh ethyl acetate was done and the procedure followed from this point on as described, recoveries were 93% or better. The reproducibility of the determination of aliquots of the same sample was to ±5.0%.

The recoveries from soil that contained 40 and 200 μg. of calcium cyanamide per gram of sample were 95 and 98%, respectively. However, if the amount of cyanamide present is below 20 μg. per gram of soil and the acid filtrate is highly pigmented, it may be difficult to determine directly. In such cases an ethyl acetate extract similar to that used for the urine would perhaps be useful.

When the paper chromatographic system described by Milks and Janes [7] was used in our laboratory, 5 to 10 μg. of cyanamide produced a clearly visible red color when the paper was sprayed with a solution of 0.2*M* $Na_3[Fe(CN)_5NH_3]$ dissolved in the pH 10.5 carbonate buffer.

**Table I. Conditions for Optimum Color Development of the Reaction of Cyanamide with Pentacyanoammineferrate**

| pH | Reaction Time, Min. | 0.2*M* $Na_3[Fe(CN)_5NH_3]$, Ml. | Absorbance at 530 Mμ in Presence of 40 μg. $CaCN_2$ |
|---|---|---|---|
| 5.0 | 30 | 0.2 | 0.08 |
| 7.0 | 30 | 0.2 | 0.15 |
| 8.5 | 30 | 0.2 | 0.32 |
| 9.5 | 30 | 0.2 | 0.45 |
| 10.0 | 30 | 0.2 | 0.50 |
| 10.5 | 30 | 0.2 | 0.52 |
| 11.0 | 30 | 0.2 | 0.50 |
| 10.5 | 5 | 0.2 | 0.38 |
| | 10 | 0.2 | 0.48 |
| | 20 | 0.2 | 0.50 |
| | 30 | 0.2 | 0.52 |
| | 60 | 0.2 | 0.52 |
| | 300 | 0.2 | 0.50 |
| | 30 | 0.05 | 0.48 |
| | | 0.10 | 0.50 |
| | | 0.15 | 0.52 |
| | | 0.20 | 0.52 |

## DISCUSSION

Buchanan and Barsky (4) and Baudisch (3) reported that ferrocyanide solutions formed a red complex with cyanamide. This reaction was shown to be light-dependent and was postulated (4) to result from the following mechanism.

$$[Fe(CN)_6]^{-4} \underset{\text{dark}}{\overset{\text{light}}{\rightleftharpoons}} [Fe(CN)_5]^{-3} + CN^- \quad (1)$$

$$[Fe(CN)_5]^{-3} + H_2NCN \rightleftharpoons [Fe(CN)_5H_2NCN]^{-3} \quad (2)$$

Deep red color

Atmospheric oxygen was thought to play no role in the formation of the complex. Baudisch (3) confirmed the photosensitivity but attributed the reaction to result as follows:

$$[Fe(CN)_6]^{-4} + H_2O \xrightarrow{\text{light}} [Fe(CN)_5OH]^{-4} + HCN \quad (3)$$

The pentacyano-hydroxyl complex then was thought to undergo autoxidation in air to produce an intermediate which reacts with cyanamide to give a red complex.

It was confirmed in our laboratories that aqueous solutions of ferrocyanide (but not ferricyanide) would react with cyanamide, but it was necessary first to activate ferrocyanide by a 24- to 36-hour aeration before use. In the presence of cyanamide, at an alkaline pH, these freshly prepared solutions developed a red color only when exposed to strong light. A useful quantitative method of analysis was based on these observations. However, the inconvenience and the difficulty of reproducing the reagent left something to be desired. The resemblance of the pentacyanoammineferrate, $Na_3[Fe(CN)_5NH_3]$, to the intermediate postulated in Equation 3 and the availability of this compound prompted the attempt to substitute it for the light- and air-activated ferrocyanide. This work resulted in the quantitative assay procedure as described.

After the completion of this work, Fearon's description (6) of a qualitative test for guanidines, urea, and thiourea came to the attention of the authors. This worker studied a variety of compounds containing an amidine or related functional groups and listed cyanamide as slowly yielding an orange-red color when in contact with a solution of freshly prepared pentacyanoammineferrate. Although no effort was made to adapt this finding to a quantitative method of analysis, Fearon must be credited as among the first to indicate the potential utility of pentacyanoammineferrate as an analytical reagent.

In view of the requirement for light and air before ferrocyanide will react with cyanamide, the effects of these two variables were studied on the development of color when the pentacyanoammineferrate reagent was used.

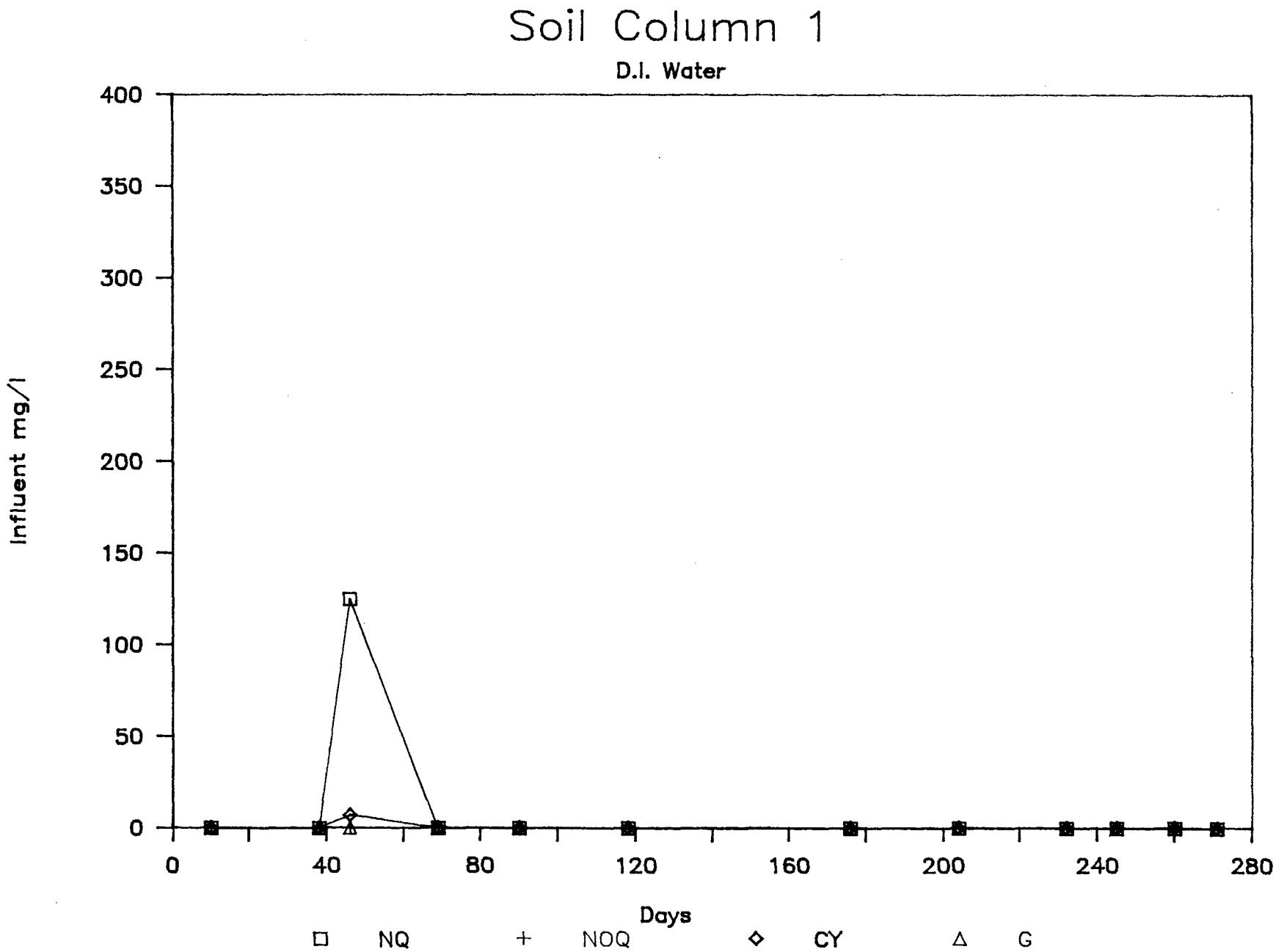
Solutions of all the reactants were prepared in water that previously had been boiled and cooled under nitrogen

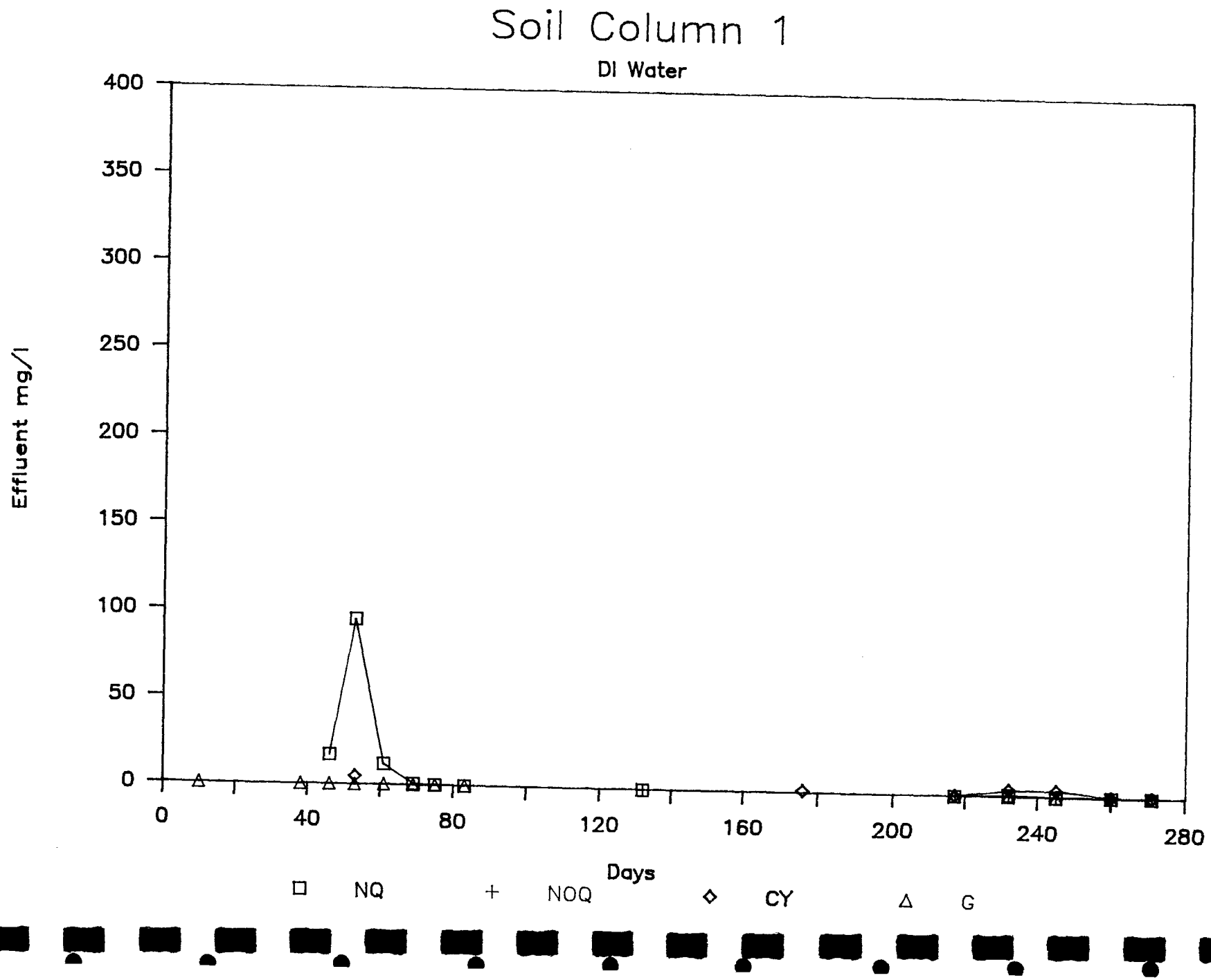
**Table II. Effect of Various Compounds on Development of the Cyanamide-Pentacyanoammineferrate Colored Complex**

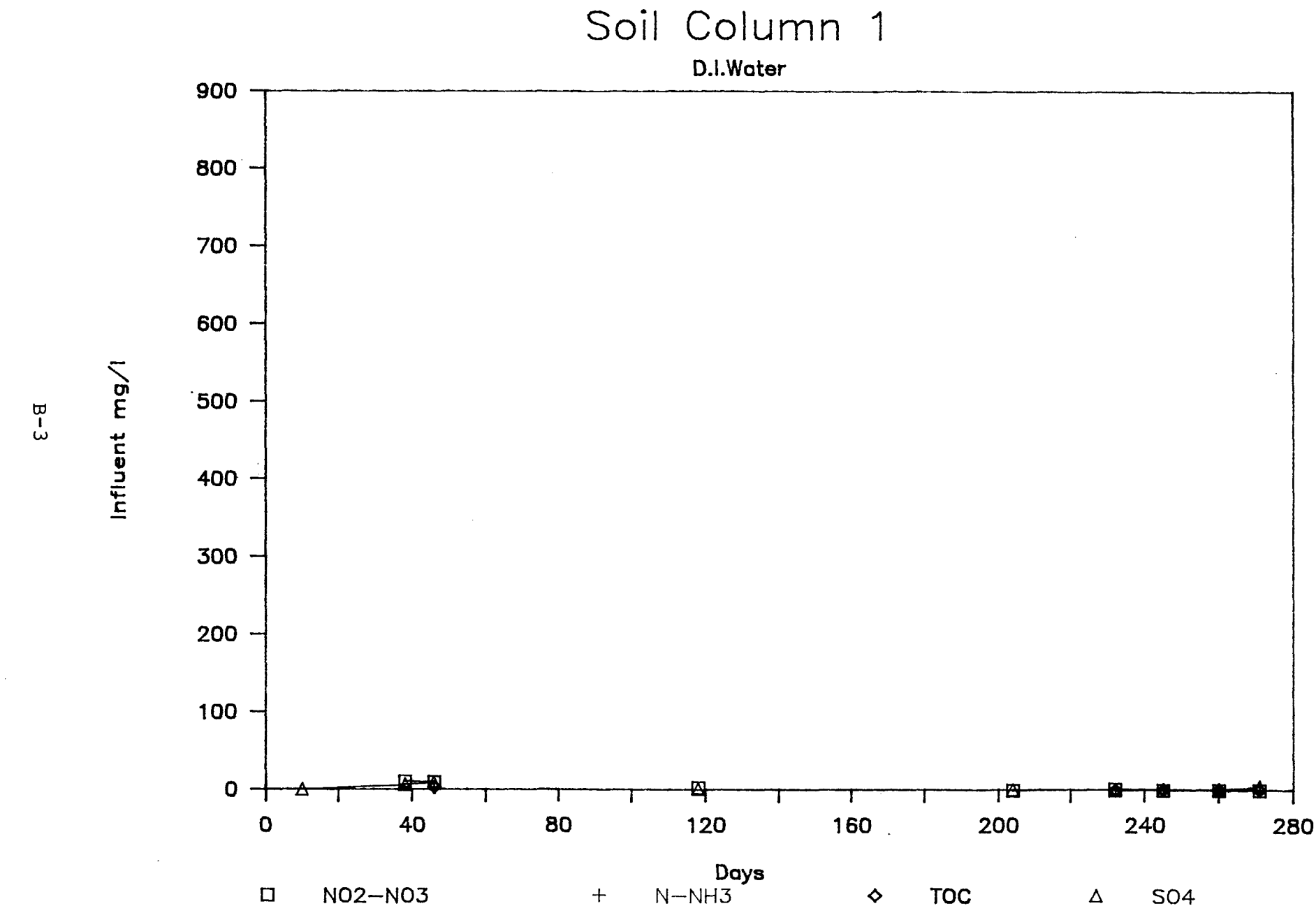
| | Assayed, μg. per Tube | Absorbance at 530 Mμ Alone | Absorbance at 530 Mμ Plus 40 μg. $CaCN_2$ |
|---|---|---|---|
| Calcium cyanamide | 40 | 0.51 | ... |
| Cyanamide | 21 | 0.51 | ... |
| Urea | 200 | −0.02 | 0.50 |
| Ammonia | 200 | −0.01 | 0.50 |
| Dicyandiamide | 200 | 0.07 | 0.58 |
| Melamine | 200 | −0.02 | 0.50 |
| Guanidine | 200 | 0.05 | 0.52 |
| Cyanide | 200 | −0.02 | 0.50 |
| Citric acid | 200 | 0.00 | 0.51 |

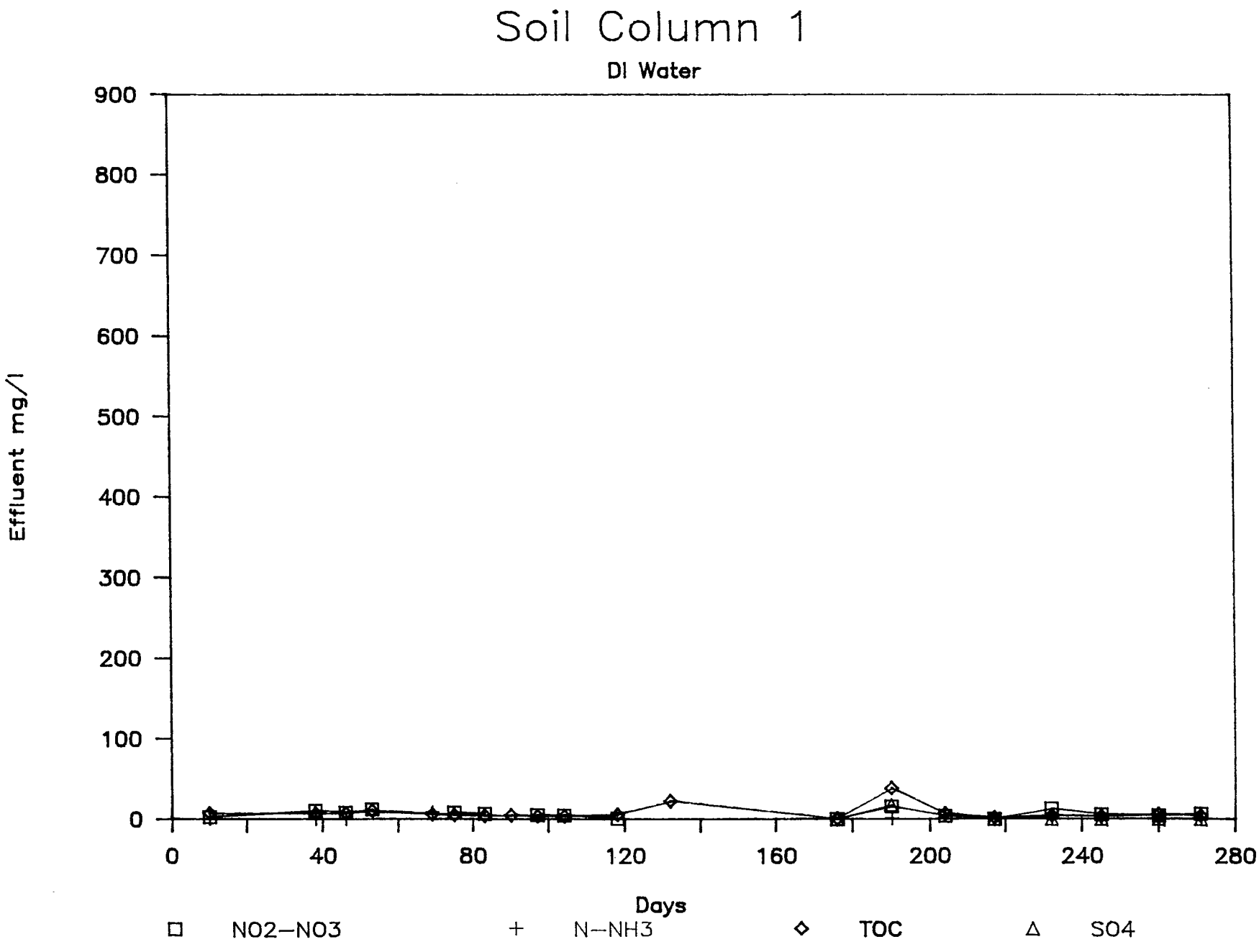
# APPENDIX B

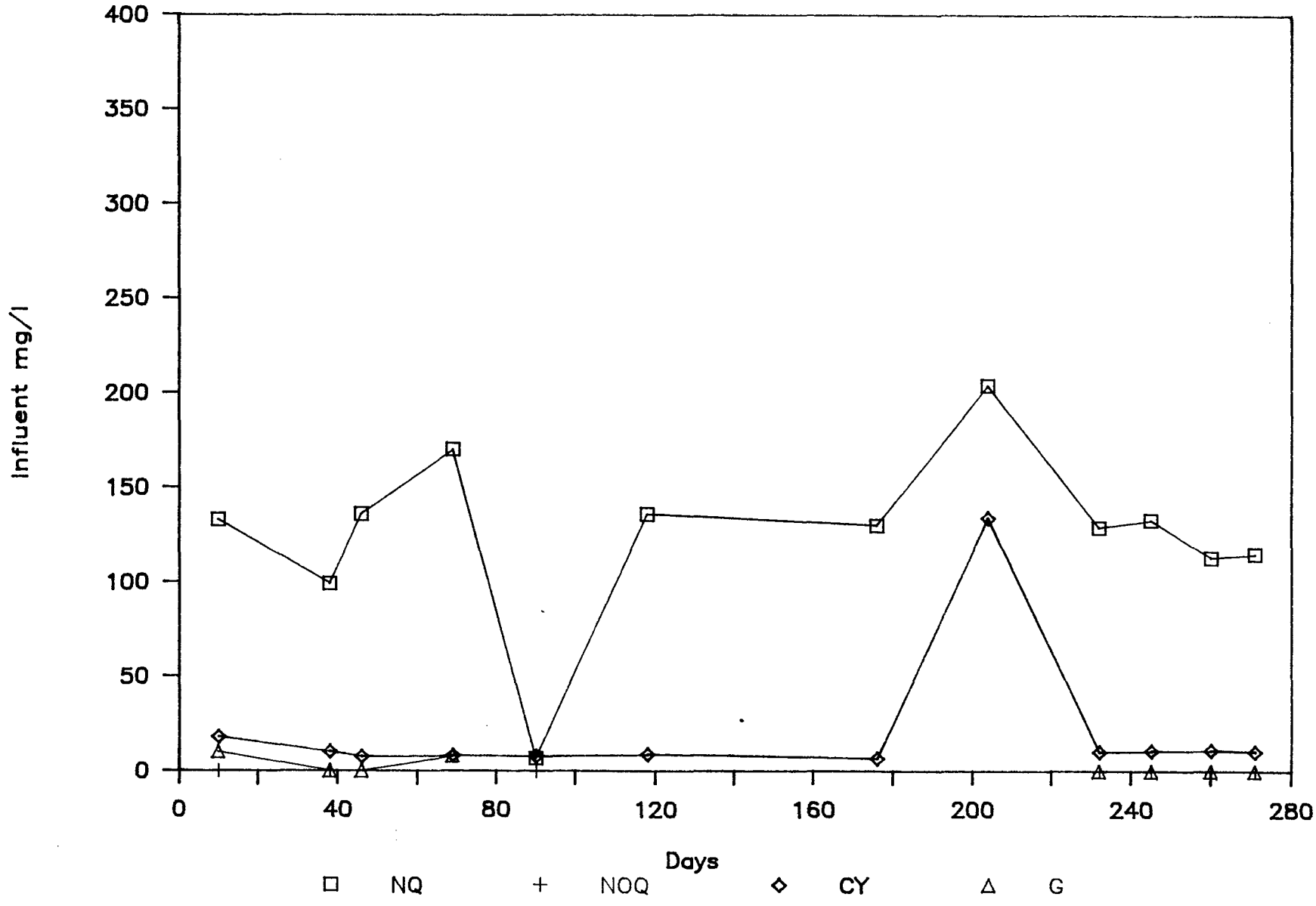
## CONTINUOUS FLOW SOIL COLUMN - GRAPHICS


Soil Column 1
D.I. Water
Influent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

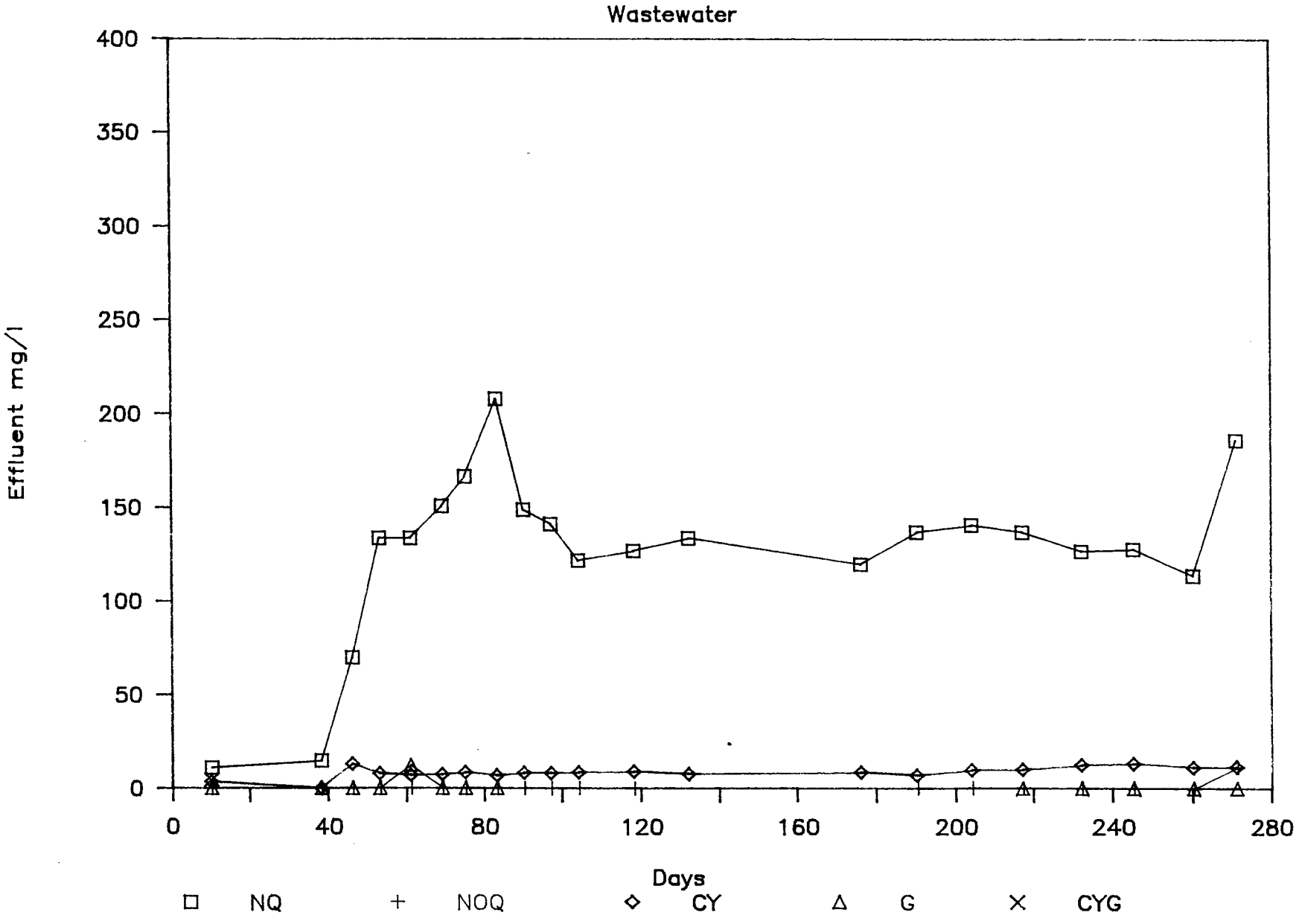

Soil Column 1
DI Water
Effluent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

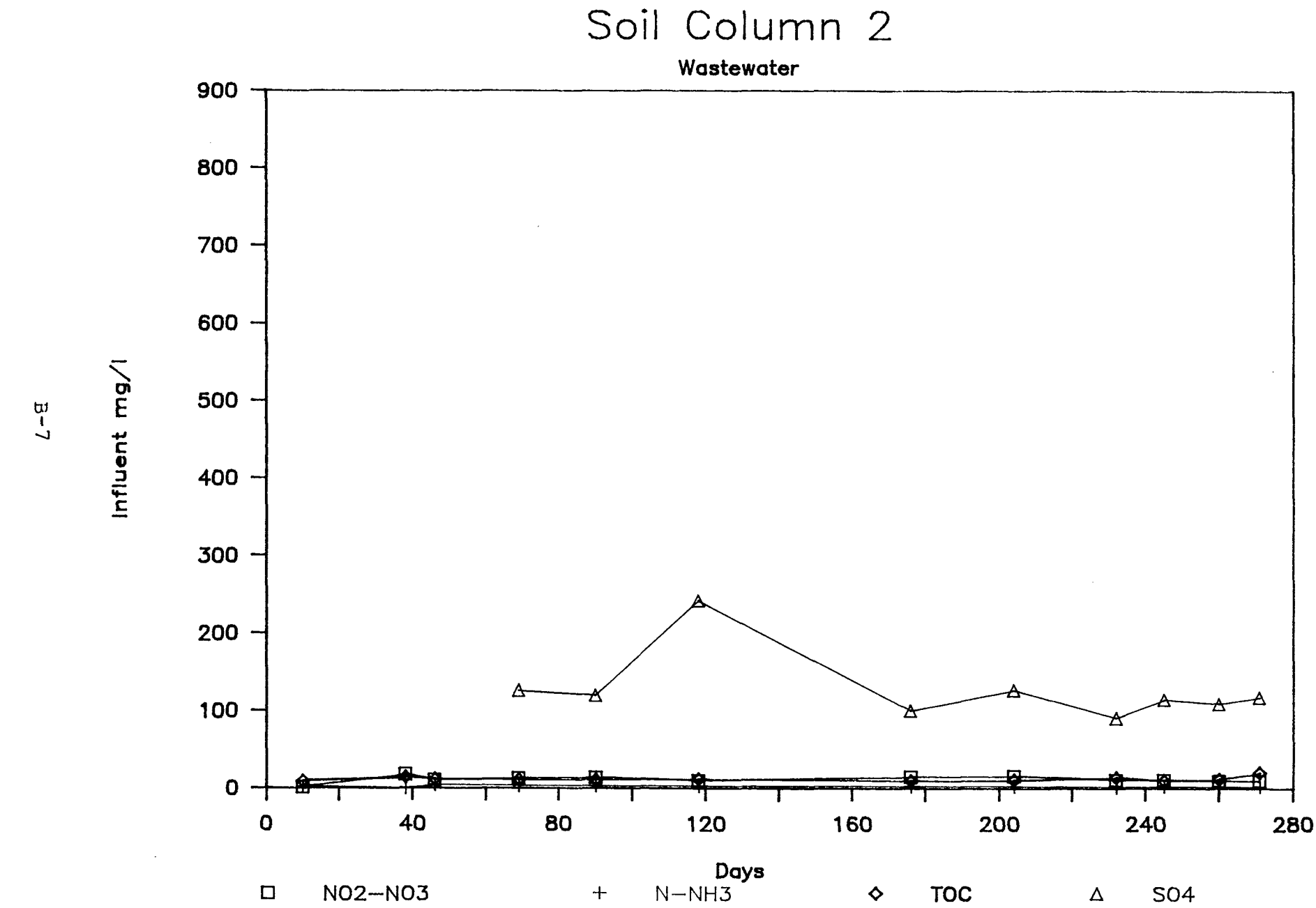

Soil Column 1
D.I.Water
900
800
700
600
500
400
300
200
100
0
Influent mg/l
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4

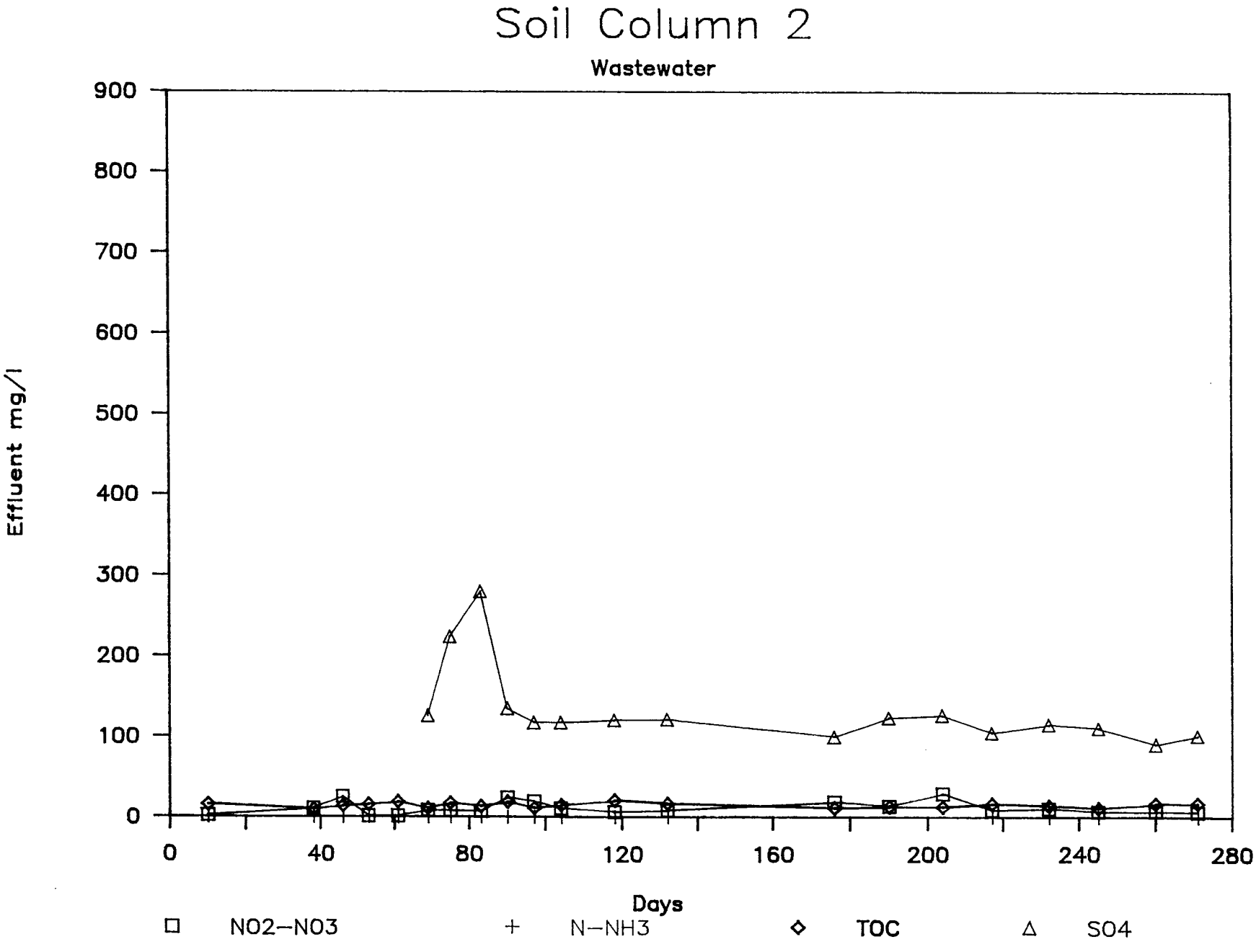

Soil Column 1
DI Water
900
800
700
600
500
400
300
200
100
0
Effluent mg/l
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4

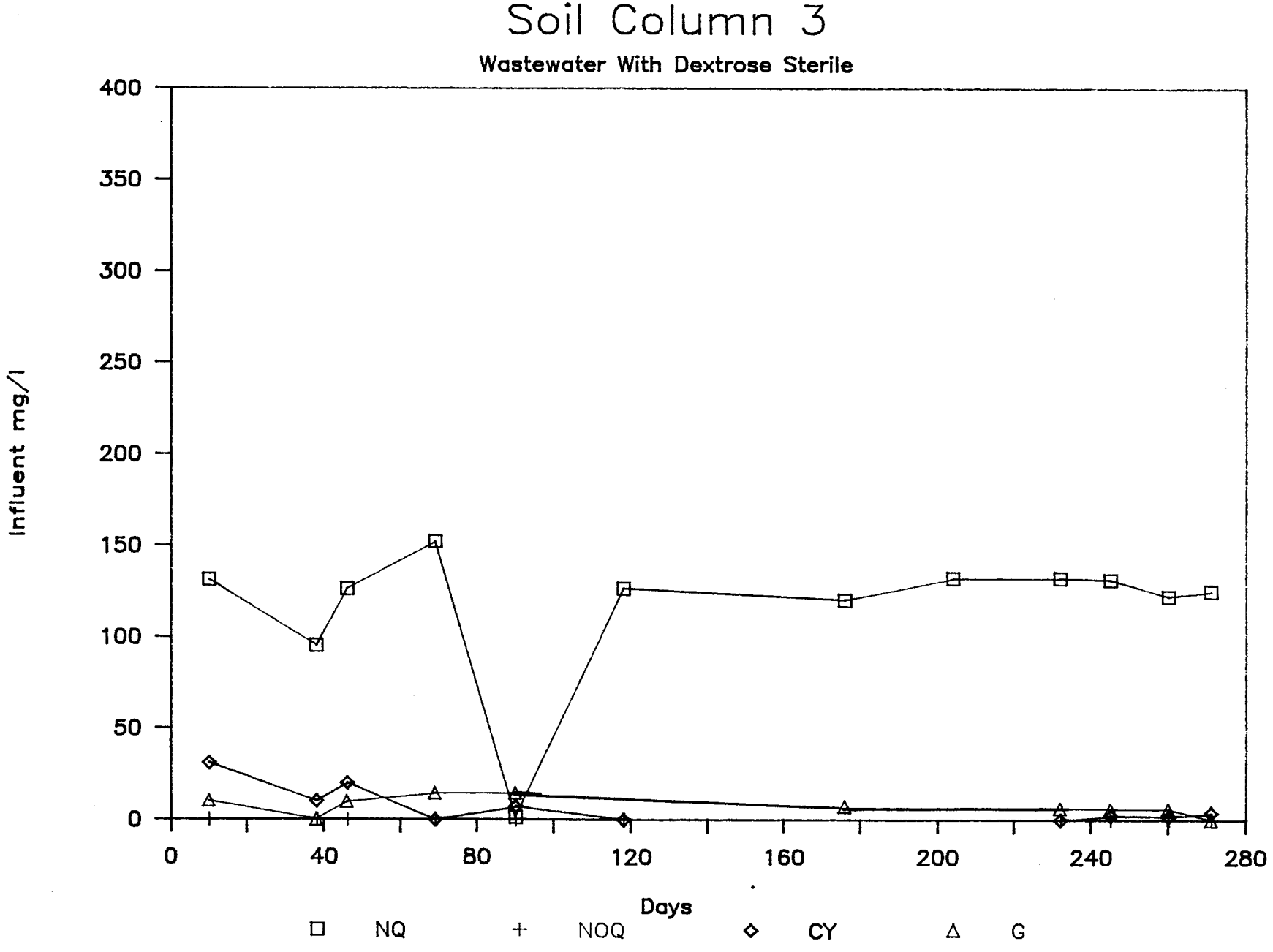

Soil Column 2
Wastewater
400
350
300
250
200
150
100
50
0
Influent mg/l
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

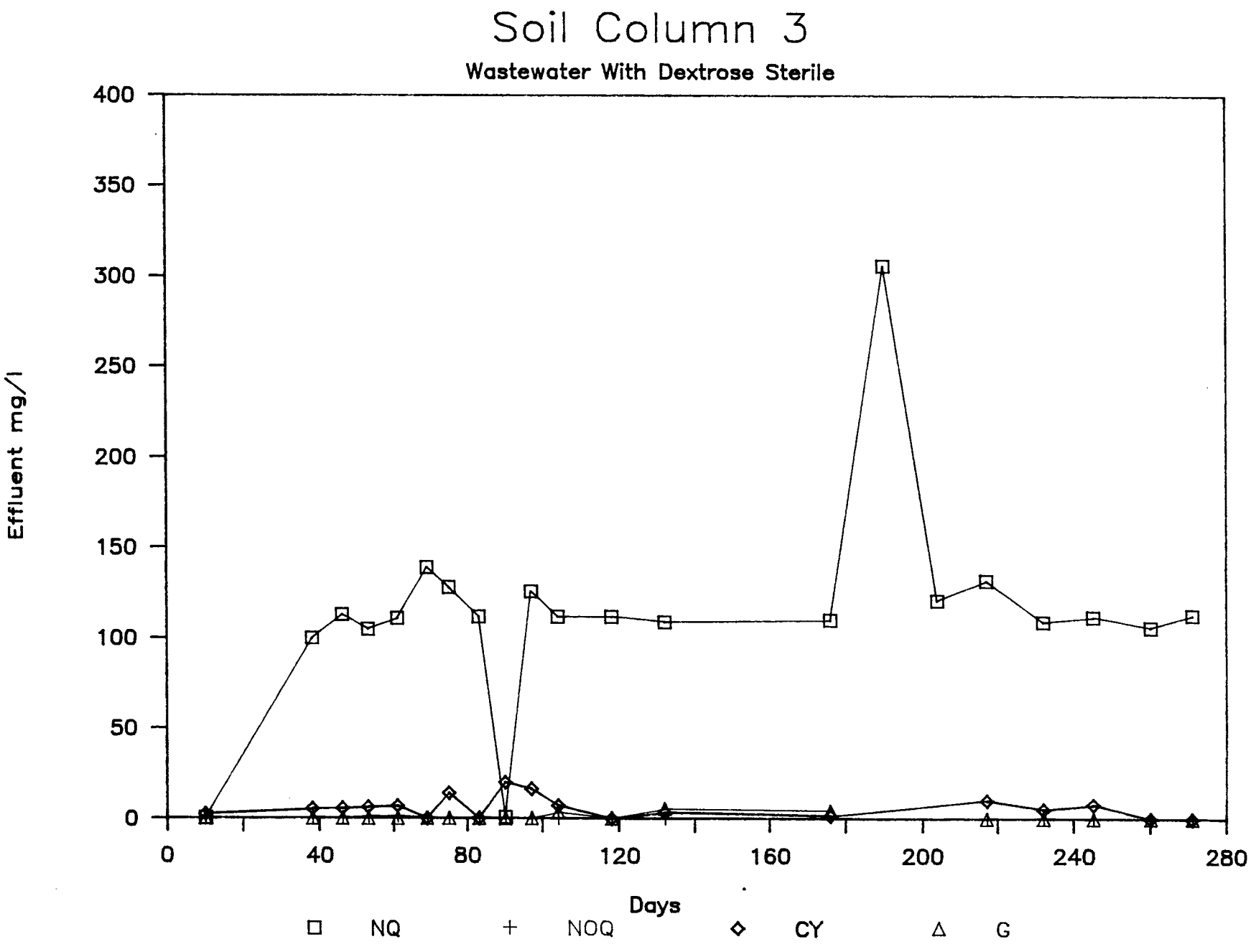
# Soil Column 2

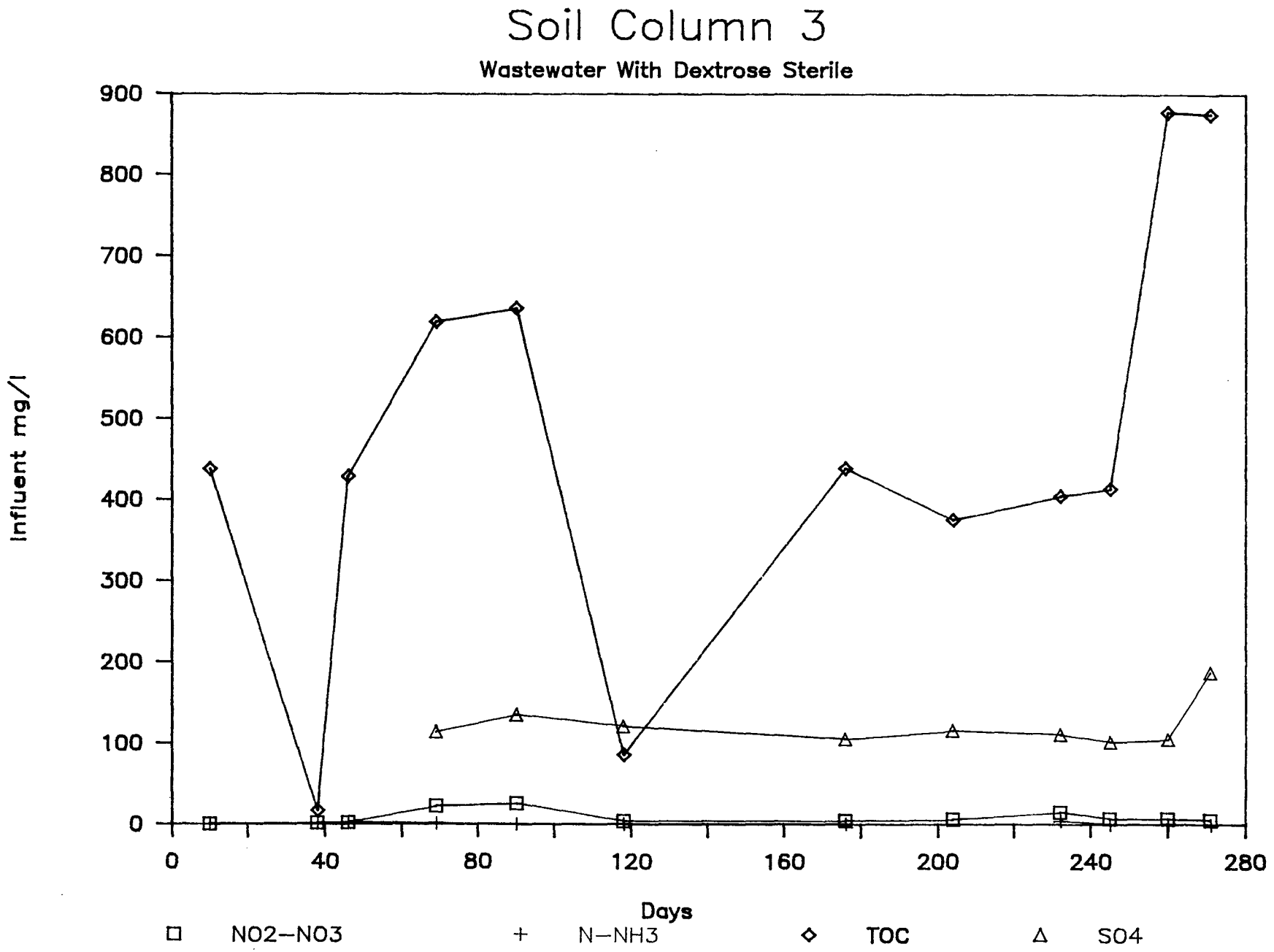


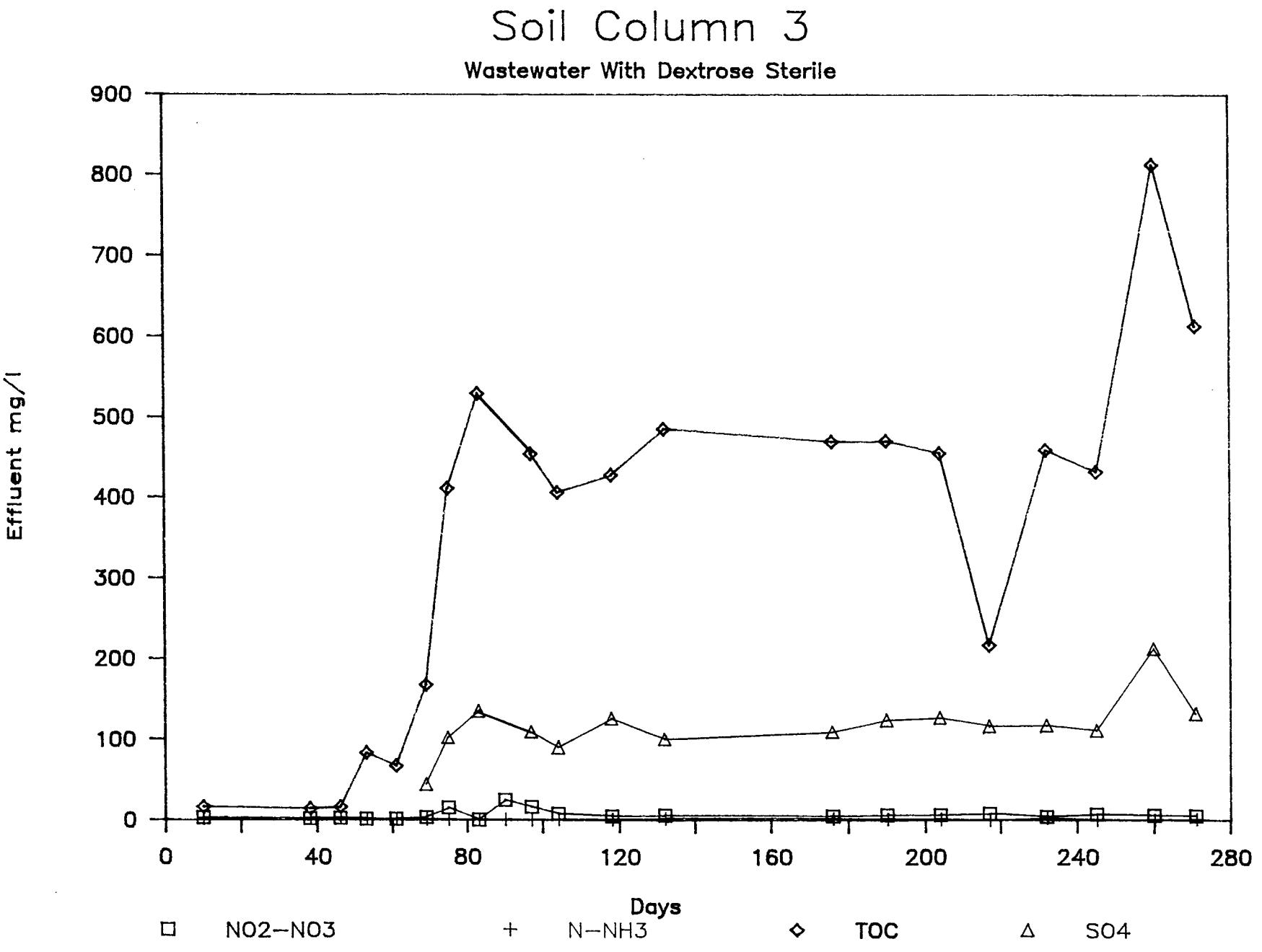

Soil Column 2
Wastewater
900
800
700
600
500
400
300
200
100
0
Influent mg/l
0
40
80
120
160
200
240
280
Days
NO2-NO3
N-NH3
TOC
SO4

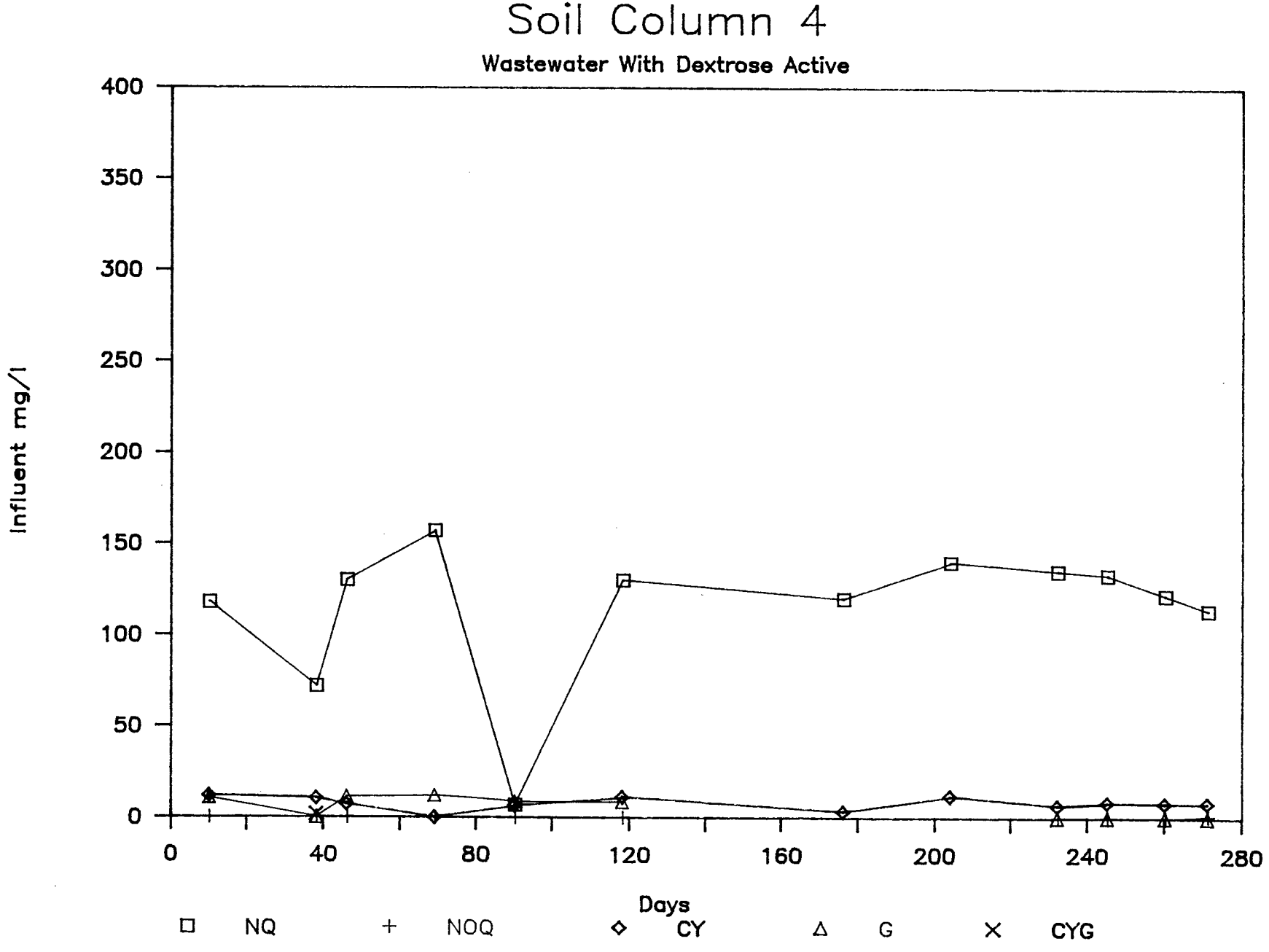

Soil Column 2
Wastewater
900
800
700
600
500
400
300
200
100
0
Effluent mg/l
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4

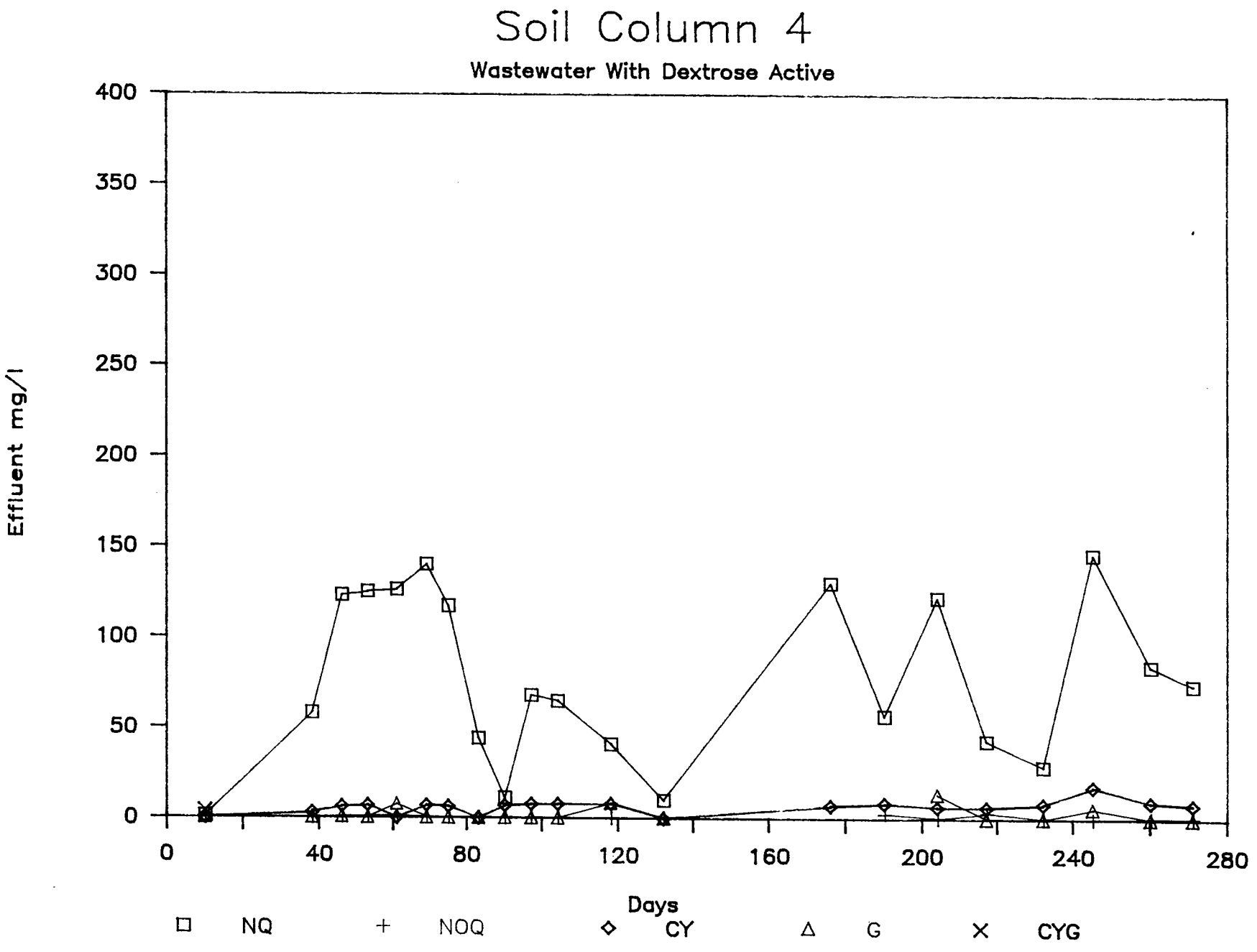

Soil Column 3
Wastewater With Dextrose Sterile
Influent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

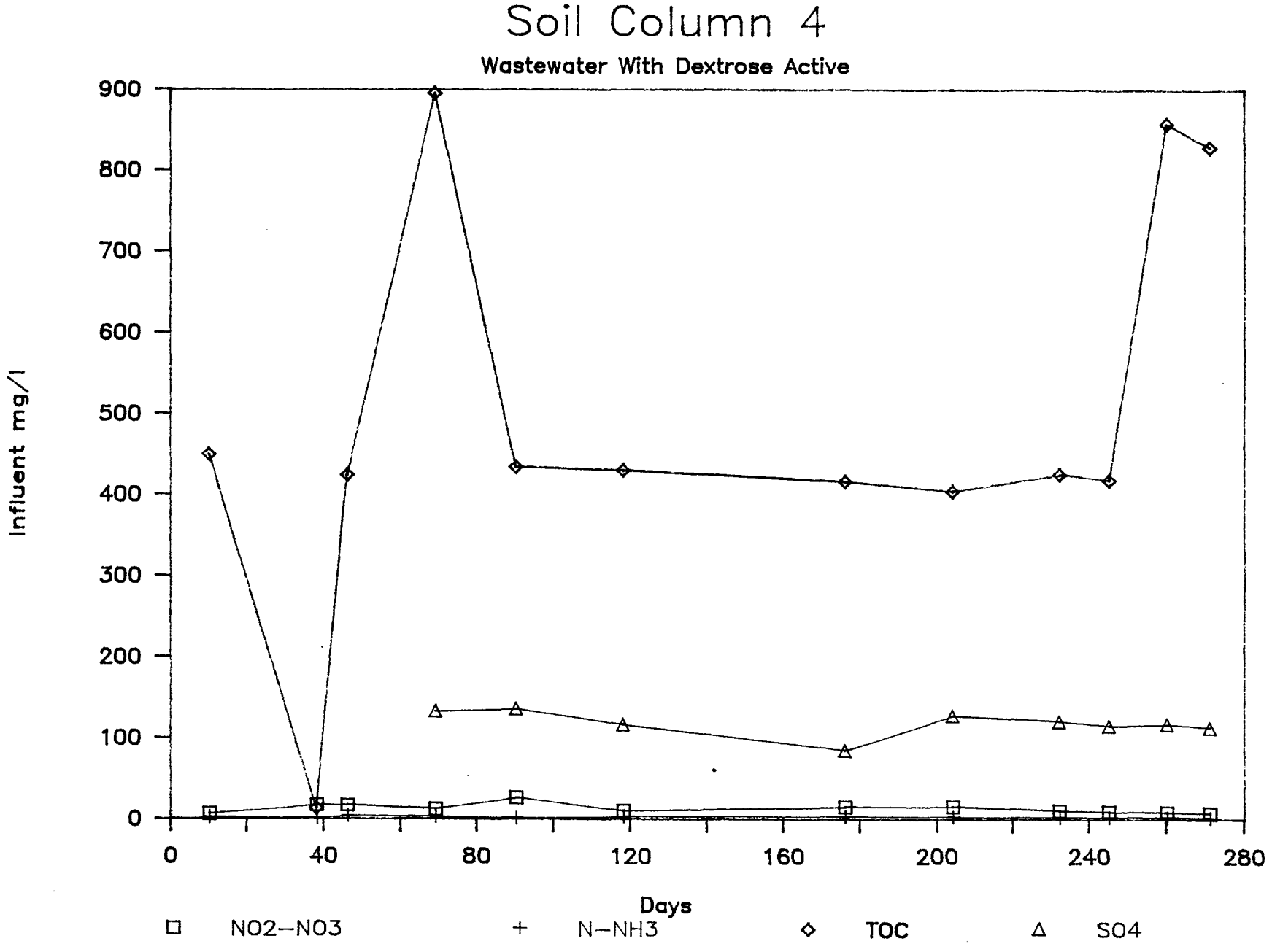

Soil Column 3
Wastewater With Dextrose Sterile
Effluent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

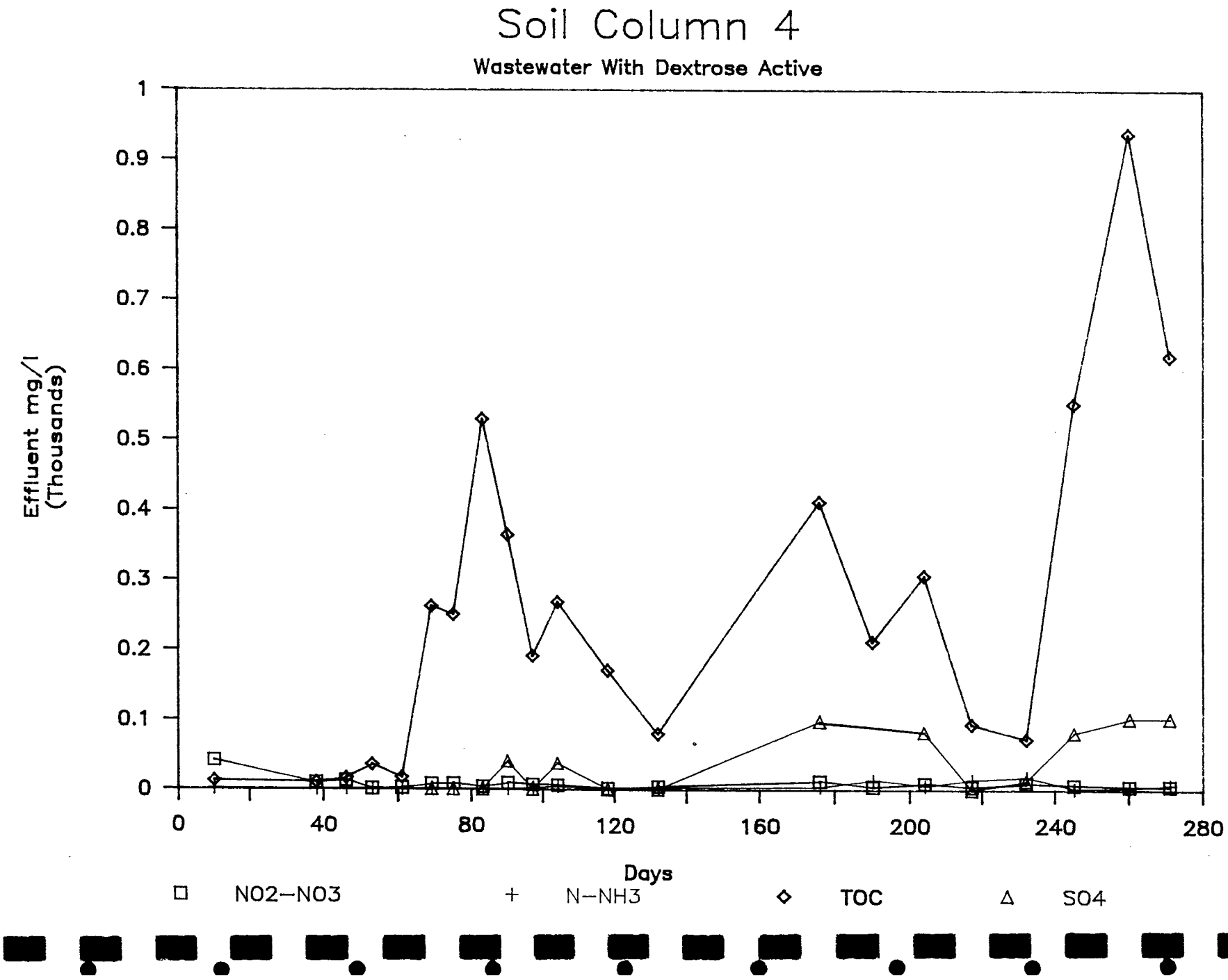

Soil Column 3
Wastewater With Dextrose Sterile
Influent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4

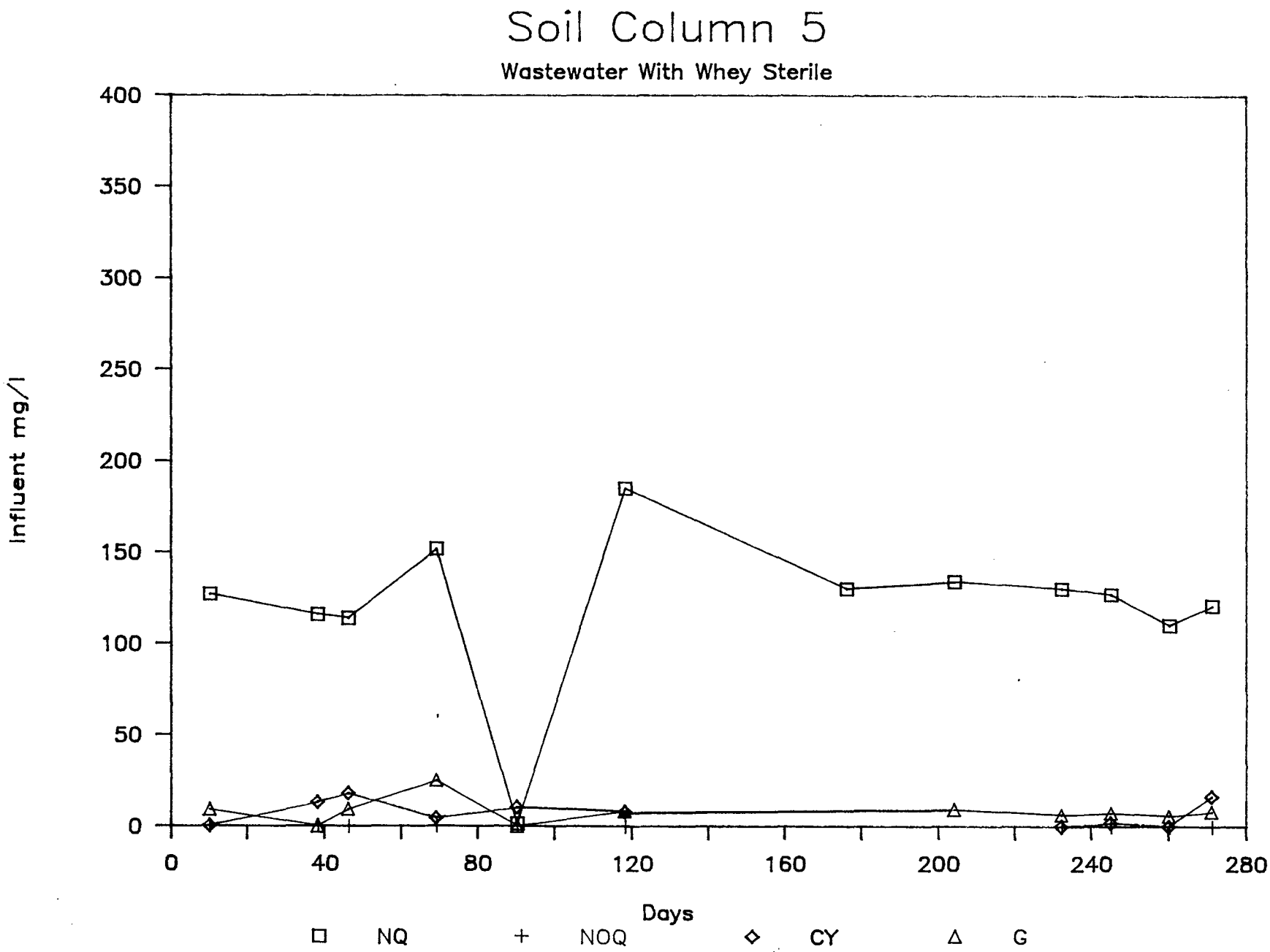

Soil Column 3
Wastewater With Dextrose Sterile
Effluent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
NO2-NO3
N-NH3
TOC
SO4

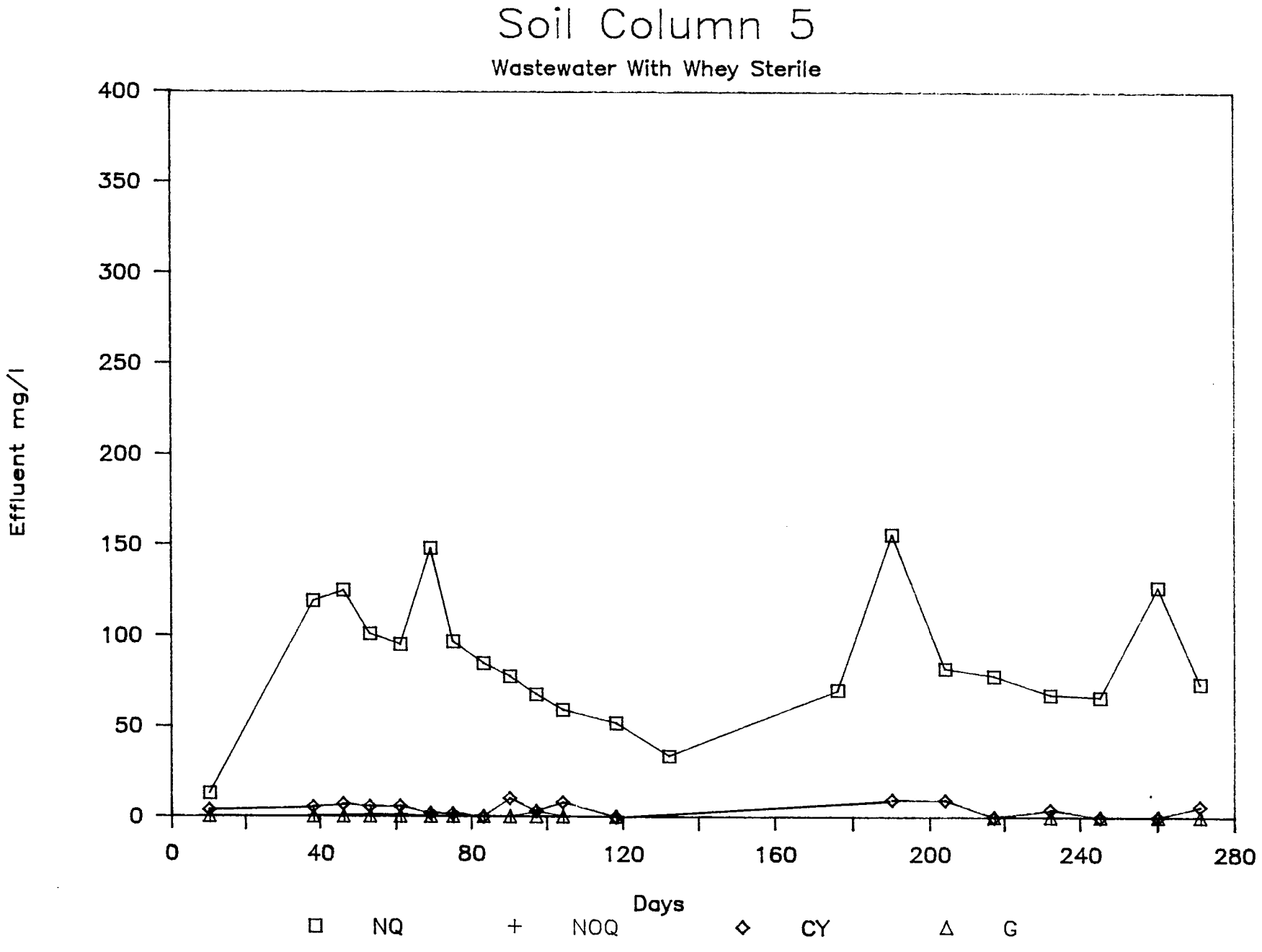

Soil Column 4
Wastewater With Dextrose Active
Influent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G
CYG

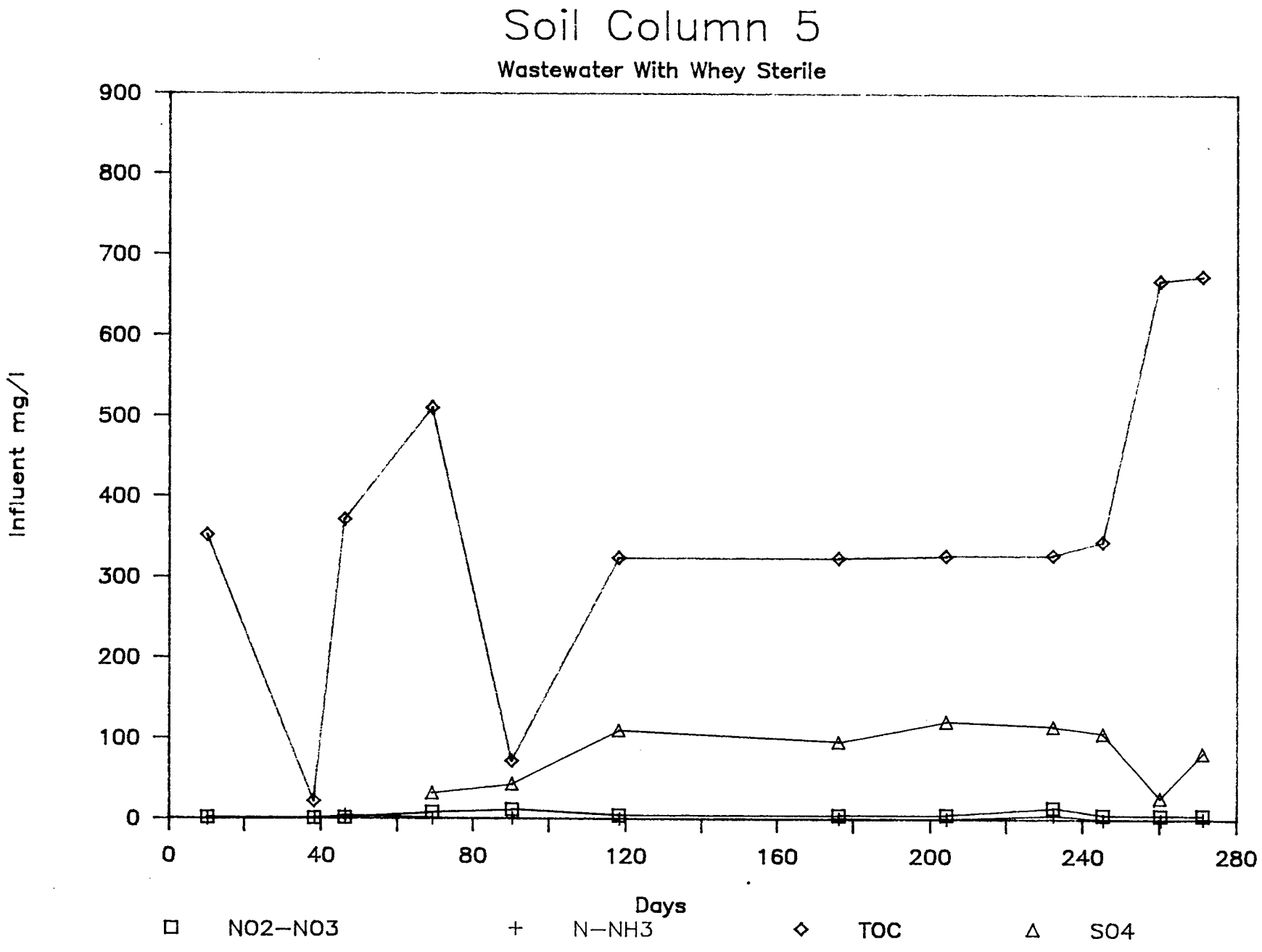

Soil Column 4
Wastewater With Dextrose Active
Effluent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G
CYG

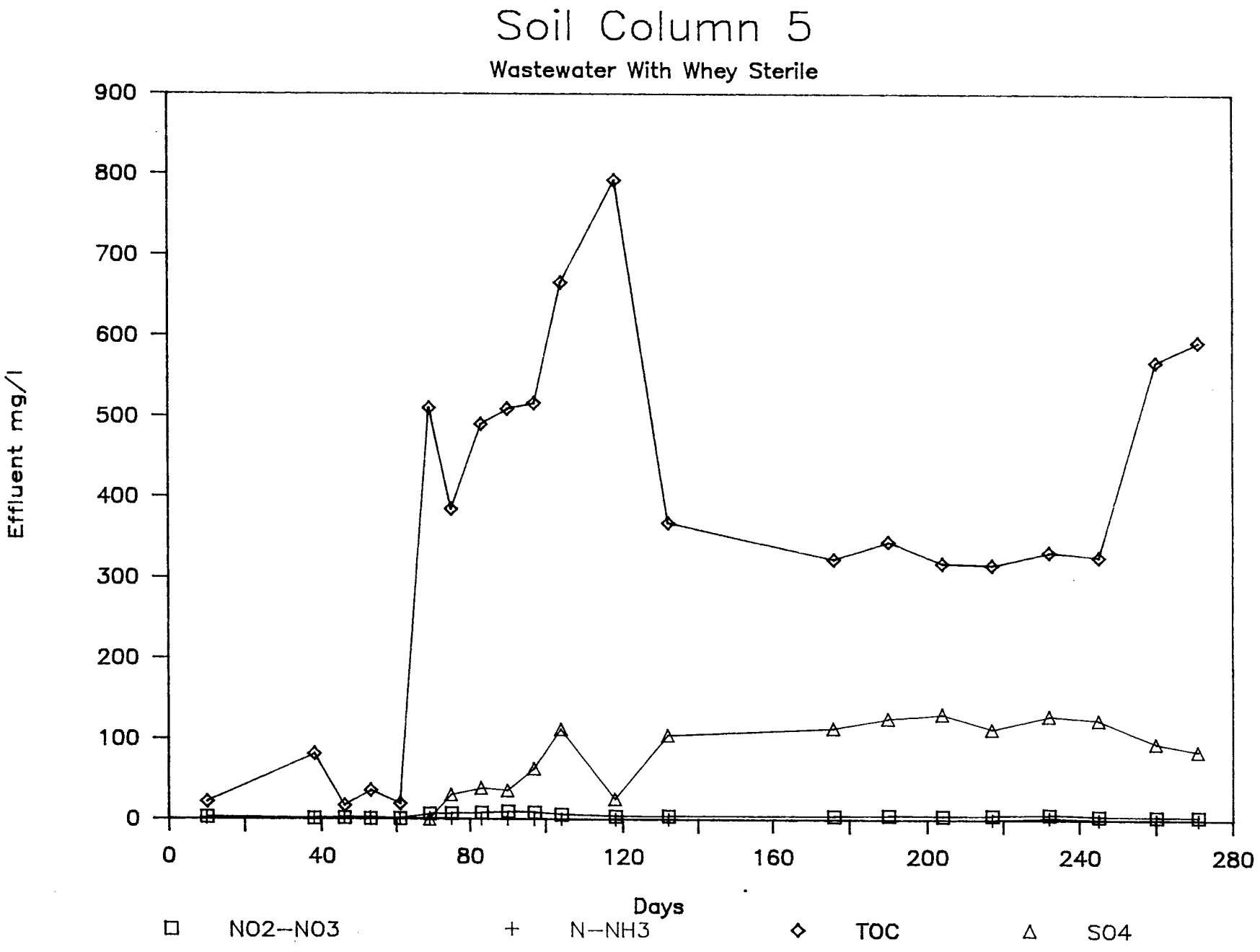

Soil Column 4
Wastewater With Dextrose Active
Influent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4

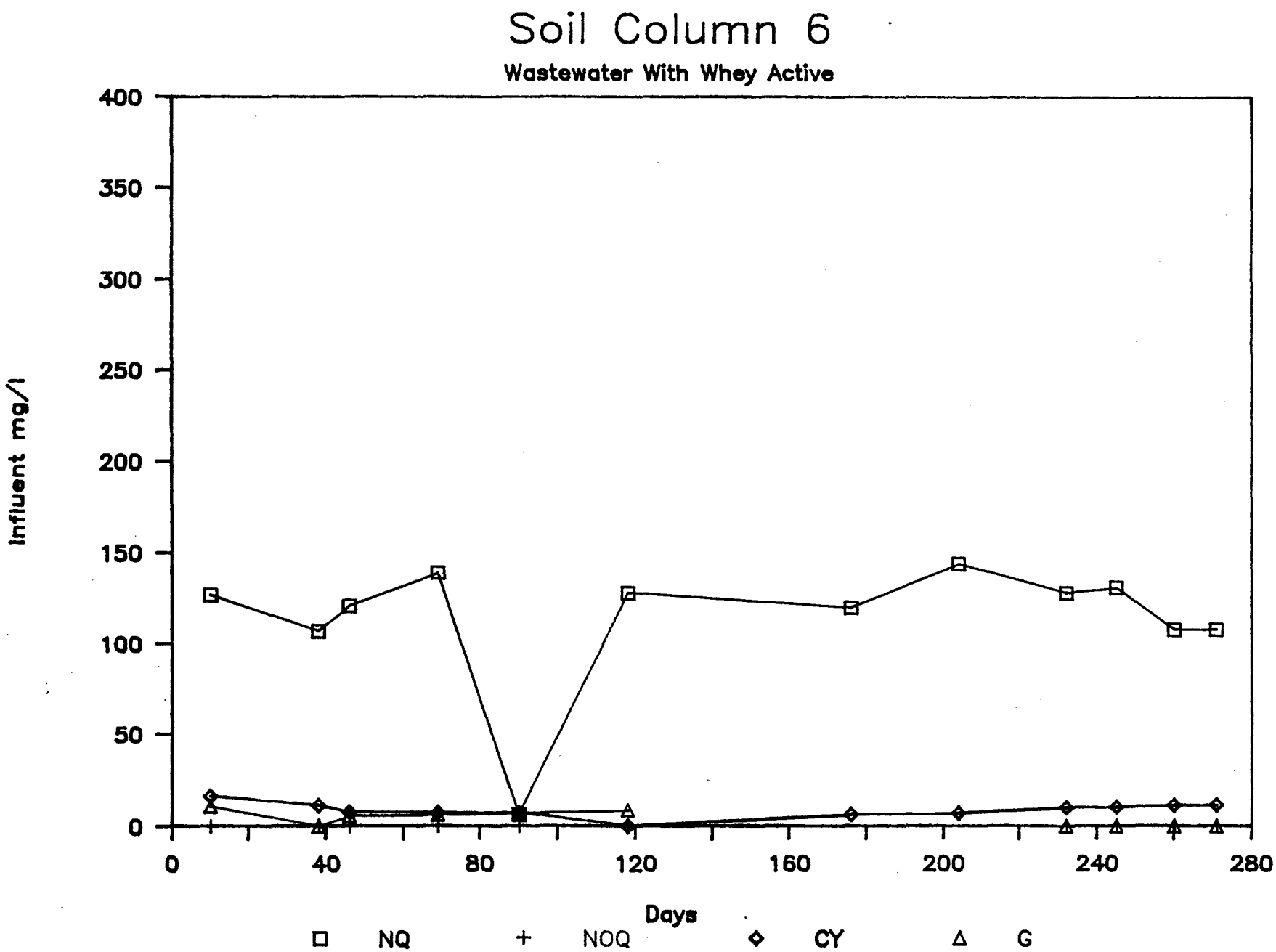

Soil Column 4
Wastewater With Dextrose Active
Effluent mg/l
(Thousands)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
40
80
120
160
200
240
280
Days
NO2-NO3
N-NH3
TOC
SO4

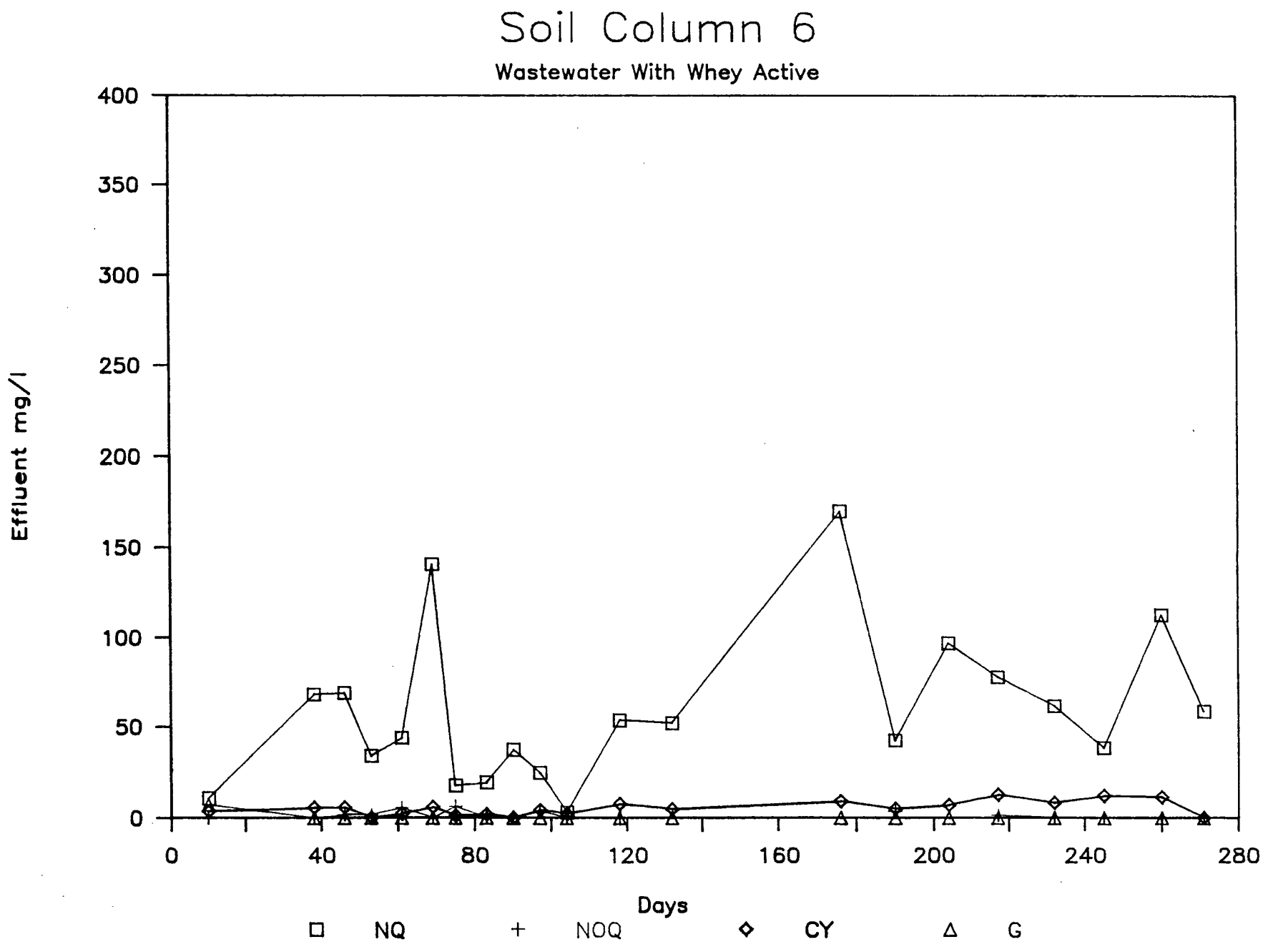

Soil Column 5
Wastewater With Whey Sterile
Influent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

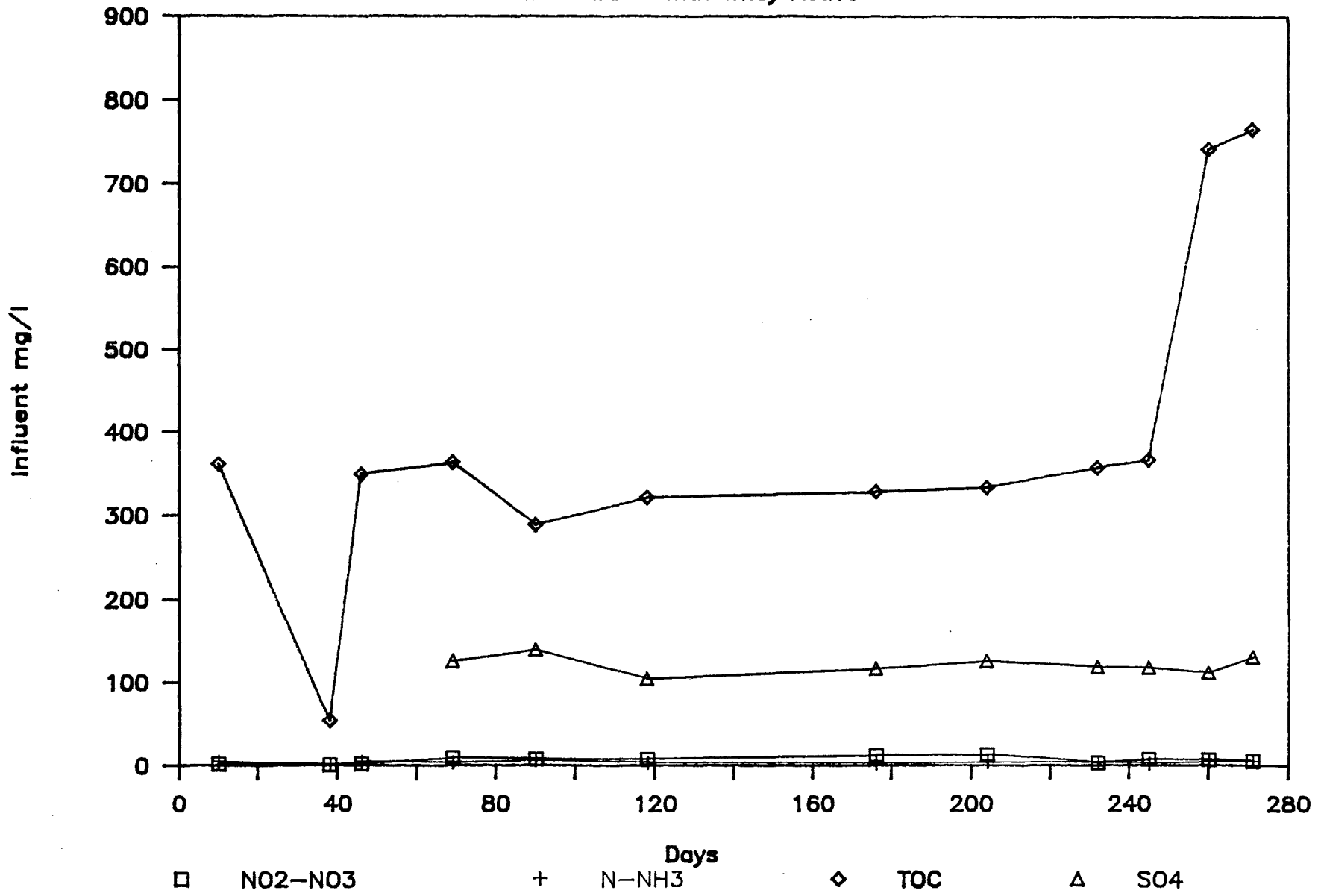

Soil Column 5
Wastewater With Whey Sterile
Effluent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G

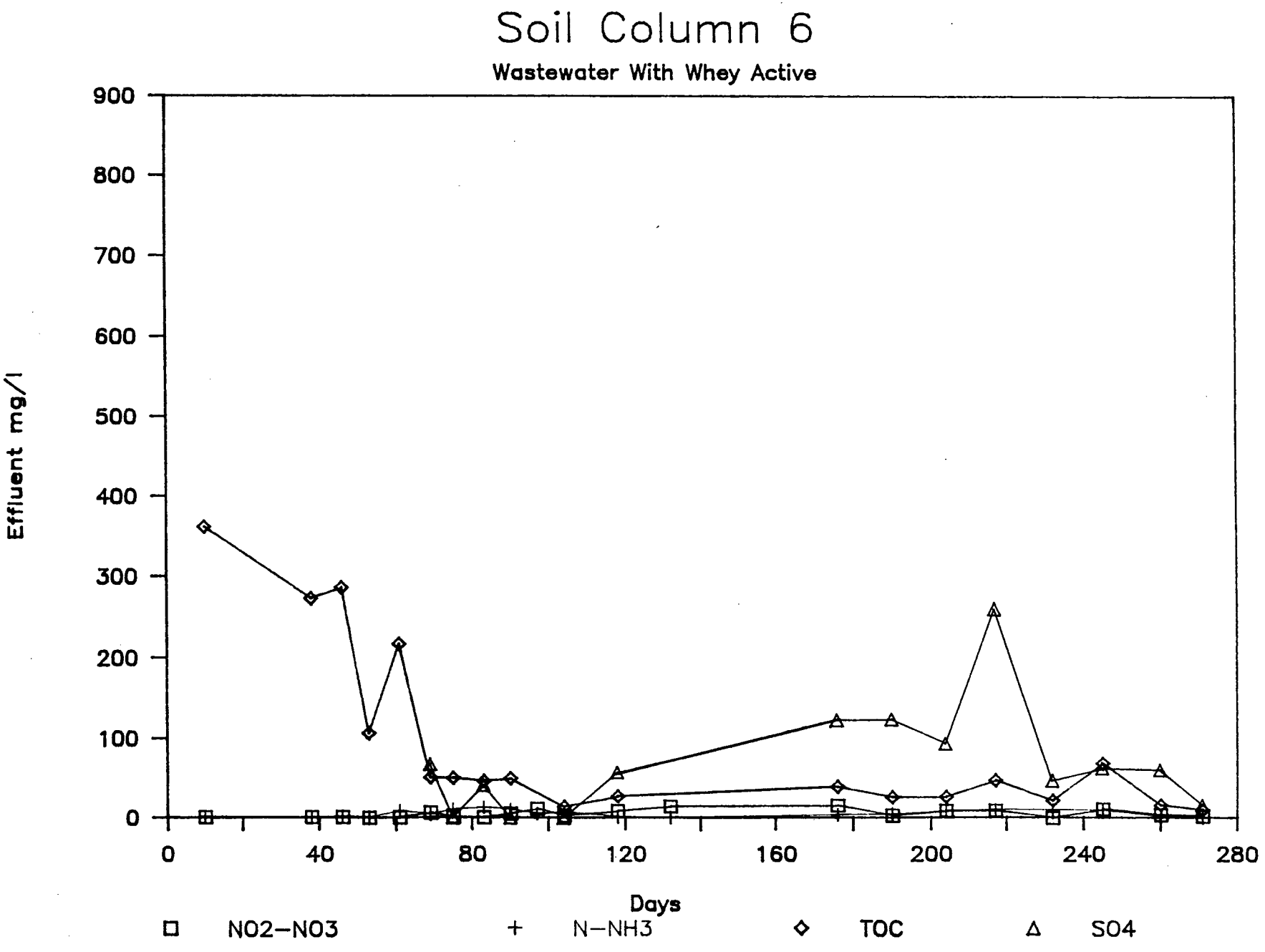

Soil Column 5
Wastewater With Whey Sterile
900
800
700
600
500
400
300
200
100
0
Influent mg/l
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4


Soil Column 5
Wastewater With Whey Sterile
Effluent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
NO2--NO3
N--NH3
TOC
SO4


Soil Column 6
Wastewater With Whey Active
Influent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G


Soil Column 6
Wastewater With Whey Active
Effluent mg/l
400
350
300
250
200
150
100
50
0
0
40
80
120
160
200
240
280
Days
NQ
NOQ
CY
G


Soil Column 6
Wastewater With Whey Active
900
800
700
600
500
400
300
200
100
0
Influent mg/l
0
40
80
120
160
200
240
280
Days
NO2–NO3
N–NH3
TOC
SO4


Soil Column 6
Wastewater With Whey Active
Effluent mg/l
900
800
700
600
500
400
300
200
100
0
0
40
80
120
160
200
240
280
Days
NO2-NO3
N-NH3
TOC
SO4

# APPENDIX C

## CONTINUOUS FLOW SOIL COLUMN - DATA TABLE

Continuous Flow SFAAP Soil Columns

Soil Column 1
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NQ | 0.00 | 0.00 | 125.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NOQ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 0.00 | 0.00 | 7.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NO2-NO3 | 0.00 | 10.60 | 10.30 | 0.00 | 0.00 | 2.90 | 0.00 | 0.20 | 1.60 | 0.00 | 0.00 | 0.00 |
| NH3-N | 0.00 | 0.00 | 2.92 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 | 0.00 |
| TOC | 0.52 | 6.42 | 9.80 | 0.00 | 0.00 | 1.67 | 0.00 | 1.15 | 0.00 | 0.00 | 0.98 | 5.17 |
| SO4 | | | | | | | | | 0.00 | 0.00 | 0.00 | 0.00 |

Soil Column 1
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NQ | 0.00 | 0.00 | 17.00 | 95.00 | 12.00 | 0.70 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| NOQ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 0.00 | 0.00 | 0.00 | 4.80 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.80 | 0.00 | 0.00 | 0.00 | 3.50 | 3.60 | 0.00 | 0.00 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NO2-NO3 | 2.80 | 11.00 | 8.60 | 12.40 | 0.00 | 0.00 | 8.40 | 6.90 | 0.00 | 5.80 | 5.30 | 0.90 | 0.00 | 0.00 | 16.00 | 4.00 | 0.00 | 13.00 | 6.00 | 4.50 | 7.00 |
| NH3-N | 0.26 | 0.03 | 0.04 | 0.00 | 0.00 | 9.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 | 0.16 | 0.00 | 0.00 | 0.00 |
| TOC | 7.30 | 7.07 | 7.14 | 10.70 | 0.00 | 5.86 | 5.30 | 3.83 | 4.74 | 3.30 | 3.62 | 5.71 | 22.10 | 0.00 | 38.40 | 6.47 | 1.24 | 4.17 | 3.14 | 5.67 | 4.04 |
| SO4 | | | | | | | | | | | | | | 0.00 | 17.80 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Continuous Flow SFAAP Soil Columns

Soil Column 2
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NQ | 133.00 | 99.00 | 136.00 | 170.00 | 6.70 | 136.00 | 130.00 | 204.00 | 129.00 | 133.00 | 113.00 | 115.00 |
| NOQ | 0.08 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 18.00 | 10.00 | 7.50 | 8.20 | 7.60 | 8.70 | 6.50 | 134.00 | 10.00 | 10.80 | 11.00 | 10.30 |
| G | 11.70 | 0.00 | 10.50 | 7.90 | 0.00 | 7.70 | 9.10 | 9.70 | 0.00 | 0.00 | 0.00 | 0.00 |
| NO2-NO3 | 1.40 | 18.10 | 11.00 | 13.20 | 14.40 | 9.90 | 15.00 | 16.00 | 11.00 | 11.00 | 10.30 | 10.30 |
| NH3-N | 2.20 | 0.17 | 4.66 | 2.90 | 2.87 | 2.30 | 2.30 | 2.60 | 1.30 | 2.34 | 2.08 | 2.53 |
| TOC | 9.17 | 14.20 | 11.80 | 11.00 | 10.90 | 11.60 | 9.78 | 10.60 | 14.20 | 9.92 | 11.70 | 20.90 |
| SO4 | | | | 126.00 | 120.00 | 242.00 | 100.00 | 127.00 | 91.00 | 115.00 | 109.00 | 118.00 |

Soil Column 2
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NQ | 11.20 | 15.10 | 70.00 | 134.00 | 134.00 | 151.00 | 166.80 | 208.00 | 149.00 | 141.40 | 122.00 | 127.20 | 134.00 | 120.00 | 137.00 | 141.00 | 137.00 | 127.00 | 128.00 | 114.00 | 186.00 |
| NOQ | 0.02 | 0.00 | 0.22 | 0.30 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.15 | 0.00 | 0.00 | 0.00 | 0.30 | 0.45 | 0.30 | 0.00 | 0.00 | 0.00 | 11.60 |
| CY | 3.30 | 0.00 | 13.00 | 7.80 | 7.00 | 7.50 | 8.60 | 6.80 | 8.40 | 8.00 | 8.50 | 9.10 | 7.70 | 8.60 | 7.00 | 9.80 | 10.00 | 12.50 | 13.20 | 11.20 | 11.60 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 12.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CYG | | | | | | | | | | | | | | | | | | | 4.30 | | |
| NO2-NO3 | 2.40 | 11.00 | 25.70 | 1.40 | 1.60 | 8.60 | 8.10 | 7.20 | 24.60 | 19.70 | 11.00 | 6.60 | 8.80 | 19.20 | 14.00 | 30.00 | 9.00 | 11.00 | 7.00 | 6.90 | 6.80 |
| NH3-N | 0.22 | 0.00 | 0.05 | 0.00 | 0.15 | 0.13 | 0.23 | 0.09 | 0.13 | 0.00 | 0.00 | 0.00 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.24 | 0.22 | 0.00 |
| TOC | 15.80 | 9.57 | 13.60 | 15.70 | 18.60 | 10.70 | 17.30 | 13.00 | 18.50 | 11.20 | 14.90 | 20.80 | 16.70 | 11.00 | 13.20 | 12.50 | 16.50 | 14.00 | 10.90 | 16.70 | 17.10 |
| SO4 | | | | | | 126.00 | 224.00 | 280.00 | 135.00 | 118.00 | 118.00 | 121.00 | 122.00 | 100.00 | 124.00 | 127.00 | 105.00 | 116.00 | 111.00 | 91.00 | 102.00 |

Continuous Flow SFAAP Soil Columns

Soil Column 3
`Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NO | 131.00 | 95.00 | 126.00 | 152.00 | 1.40 | 126.40 | 120.00 | 132.00 | 132.00 | 131.00 | 122.00 | 125.00 |
| NOO | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 31.00 | 10.00 | 20.00 | 0.00 | 7.30 | 0.00 | 0.00 | 0.00 | 0.00 | 2.50 | 2.00 | 4.00 |
| G | 10.10 | 0.00 | 9.70 | 14.30 | 0.00 | 10.20 | 7.10 | 7.60 | 6.30 | 5.80 | 6.10 | 0.00 |
| | | | | | | | | | | | | |
| NO2-NO3 | 0.30 | 1.50 | 1.90 | 22.00 | 25.60 | 4.00 | 4.80 | 6.00 | 15.00 | 7.00 | 6.70 | 5.60 |
| NH3-N | 0.00 | 1.71 | 2.75 | 1.10 | 0.50 | 0.60 | 0.50 | 0.00 | 4.46 | 0.00 | 0.00 | 0.00 |
| TOC | 438.00 | 16.50 | 429.00 | 619.00 | 636.00 | 86.10 | 439.00 | 375.00 | 405.00 | 414.00 | 878.00 | 875.00 |
| SO4 | | | | 114.00 | 135.00 | 121.00 | 105.00 | 116.00 | 111.00 | 102.00 | 105.00 | 188.00 |

Soil Column3
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NO | 0.40 | 100.00 | 113.00 | 105.00 | 111.00 | 139.00 | 128.10 | 112.00 | 0.90 | 125.80 | 112.00 | 112.00 | 108.90 | 110.00 | 306.00 | 121.00 | 132.00 | 109.00 | 112.00 | 106.00 | 113.00 |
| NOO | 0.00 | 0.20 | 0.16 | 0.80 | 1.10 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 2.40 | 5.50 | 5.70 | 6.40 | 7.00 | 0.00 | 14.10 | 0.00 | 20.10 | 16.30 | 7.20 | 0.00 | 3.30 | 1.60 | 0.00 | 0.00 | 10.00 | 5.20 | 7.80 | 0.00 | 0.00 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.40 | 0.00 | 5.50 | 4.20 | 0.00 | 3.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | | | | | | | | | | | | | | | | |
| NO2-NO3 | 2.40 | 1.50 | 2.00 | 0.80 | 1.10 | 2.90 | 15.40 | 0.20 | 25.00 | 16.10 | 7.40 | 4.70 | 5.40 | 4.80 | 6.00 | 6.00 | 8.00 | 4.00 | 7.00 | 5.40 | 4.90 |
| NH3-N | 1.40 | 1.93 | 2.55 | 1.01 | 0.68 | 0.37 | 0.40 | 0.00 | 0.50 | 0.00 | 0.30 | 0.40 | 1.30 | 1.50 | 1.20 | 1.20 | 0.00 | 2.72 | 0.00 | 0.00 | 1.12 |
| TOC | 16.40 | 14.00 | 16.30 | 83.60 | 67.30 | 168.00 | 411.00 | 529.00 | 0.00 | 454.00 | 406.00 | 428.00 | 485.00 | 469.00 | 470.00 | 455.00 | 217.00 | 459.00 | 432.00 | 813.00 | 613.00 |
| SO4 | | | | | | 44.00 | 102.00 | 135.00 | 0.00 | 109.00 | 90.00 | 126.00 | 100.00 | 109.00 | 124.00 | 127.00 | 117.00 | 118.00 | 111.00 | 213.00 | 132.00 |

Continuous Flow SFAAP Soil Columns

Soil Column 4
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NO | 118.00 | 72.00 | 130.00 | 157.00 | 6.70 | 130.00 | 120.00 | 140.00 | 135.00 | 133.00 | 122.00 | 114.00 |
| NOO | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 1.30 |
| CY | 12.00 | 7.30 | 6.60 | 3.40 | 6.80 | 8.00 | 6.60 | 6.90 | 10.00 | 10.40 | 10.30 | 10.30 |
| G | 10.60 | 0.00 | 11.30 | 11.80 | 8.60 | 8.30 | 8.00 | 8.60 | 0.00 | 0.00 | 0.00 | 0.00 |
| CYG | | | | | | | | | 1.70 | 2.90 | 0.00 | 0.00 |
| NO2-NO3 | 5.80 | 16.70 | 16.70 | 11.50 | 25.80 | 9.70 | 14.70 | 15.00 | 10.00 | 9.00 | 8.10 | 7.70 |
| NH3-N | 1.30 | 0.14 | 3.45 | 2.20 | 0.50 | 2.30 | 3.20 | 2.70 | 2.25 | 1.84 | 2.15 | 2.00 |
| TOC | 449.00 | 12.50 | 424.00 | 895.00 | 434.00 | 430.00 | 416.00 | 404.00 | 425.00 | 418.00 | 858.00 | 829.00 |
| SO4 | | | | 132.00 | 135.00 | 116.00 | 84.00 | 127.00 | 120.00 | 115.00 | 117.00 | 113.00 |

Soil Column 4
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NO | 0.70 | 58.00 | 123.00 | 125.00 | 126.00 | 140.00 | 117.00 | 44.00 | 10.90 | 67.80 | 64.80 | 41.00 | 9.90 | 130.00 | 56.60 | 122.00 | 43.00 | 28.80 | 146.00 | 84.30 | 74.00 |
| NOO | 0.00 | 0.10 | 0.38 | 0.60 | 0.80 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 2.70 | 0.20 | 3.40 | 0.00 | 0.00 | 0.70 | 0.60 |
| CY | 0.00 | 2.90 | 6.30 | 7.00 | 0.00 | 6.90 | 6.20 | 0.00 | 6.87 | 7.90 | 7.90 | 8.00 | 0.00 | 7.10 | 8.50 | 6.50 | 6.50 | 8.30 | 17.90 | 9.50 | 8.20 |
| G | 0.00 | 0.00 | 0.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8.20 | 0.00 | 13.50 | 0.00 | 0.00 | 5.70 | 0.00 | 0.00 |
| NO2-NO3 | 41.00 | 9.00 | 12.50 | 0.80 | 1.80 | 7.30 | 7.60 | 3.60 | 8.80 | 6.60 | 5.80 | 1.20 | 4.20 | 12.30 | 4.00 | 9.00 | 4.00 | 9.00 | 7.00 | 4.50 | 5.00 |
| NH3-N | 0.61 | 0.09 | 0.39 | 0.04 | 0.09 | 0.96 | 0.28 | 0.00 | 0.30 | 2.00 | 3.90 | 0.70 | 2.50 | 3.80 | 14.10 | 5.50 | 14.50 | 18.70 | 1.71 | 3.09 | 7.45 |
| TOC | 12.20 | 9.98 | 16.30 | 36.10 | 17.20 | 262.00 | 250.00 | 529.00 | 364.00 | 191.00 | 268.00 | 171.00 | 80.50 | 412.00 | 212.00 | 306.00 | 94.60 | 73.50 | 552.00 | 938.00 | 620.00 |
| SO4 | | | | | | 0.00 | 0.00 | 0.00 | 40.00 | 0.00 | 37.00 | 0.00 | 0.00 | 98.00 | 0.00 | 82.80 | 0.00 | 12.40 | 82.00 | 102.00 | 102.00 |

Soil Column 5
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NO | 127.00 | 116.00 | 114.00 | 152.00 | 1.40 | 185.00 | 130.00 | 134.00 | 130.00 | 127.00 | 110.00 | 121.00 |
| NOO | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 0.16 | 13.00 | 18.00 | 4.40 | 10.30 | 8.00 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 16.60 |
| G | 9.00 | 0.00 | 9.30 | 24.90 | 0.00 | 14.50 | 8.40 | 9.20 | 6.20 | 7.40 | 5.70 | 8.00 |
| | | | | | | | | | | | | |
| NO2-NO3 | 1.90 | 1.50 | 2.00 | 8.60 | 12.30 | 5.10 | 5.70 | 6.00 | 15.00 | 6.00 | 5.70 | 6.00 |
| NH3-N | 0.16 | 1.57 | 4.53 | 1.00 | 1.20 | 0.40 | 1.40 | 1.20 | 5.81 | 0.00 | 0.00 | 1.67 |
| TOC | 352.00 | 21.80 | 371.00 | 510.00 | 71.60 | 324.00 | 324.00 | 327.00 | 328.00 | 345.00 | 669.00 | 675.00 |
| SO4 | | | | 32.00 | 43.00 | 110.00 | 96.00 | 122.00 | 116.00 | 107.00 | 27.00 | 83.00 |

Soil Column 5
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NO | 12.70 | 119.00 | 125.00 | 101.00 | 95.00 | 148.00 | 96.60 | 84.80 | 77.60 | 67.60 | 59.00 | 52.00 | 33.80 | 70.00 | 156.00 | 82.00 | 78.00 | 67.60 | 66.40 | 127.00 | 74.00 |
| NOO | 0.00 | 0.35 | 0.77 | 1.10 | 0.80 | 0.00 | 0.00 | 0.00 | 0.00 | 2.90 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 3.70 | 5.50 | 7.00 | 5.60 | 5.90 | 1.80 | 1.48 | 0.00 | 10.40 | 3.10 | 8.00 | 0.00 | 0.00 | 0.00 | 9.60 | 9.40 | 0.00 | 4.30 | 0.00 | 0.00 | 6.10 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | | | | | | | | | | | | | | | | |
| NO2-NO3 | 2.80 | 1.60 | 2.00 | 0.80 | 1.00 | 6.90 | 7.50 | 8.60 | 10.00 | 9.00 | 6.70 | 4.42 | 5.00 | 5.30 | 6.00 | 5.00 | 6.00 | 7.00 | 5.00 | 4.60 | 4.30 |
| NH3-N | 0.83 | 1.68 | 2.73 | 2.14 | 0.63 | 1.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.34 | 0.00 | 0.00 | 0.00 |
| TOC | 21.50 | 81.20 | 17.00 | 36.10 | 19.20 | 510.00 | 385.00 | 490.00 | 509.00 | 516.00 | 666.00 | 793.00 | 368.00 | 323.00 | 345.00 | 318.00 | 316.00 | 332.00 | 326.00 | 568.00 | 593.00 |
| SO4 | | | | | | 0.00 | 31.00 | 39.00 | 36.00 | 63.00 | 112.00 | 26.00 | 105.00 | 114.00 | 126.00 | 131.00 | 113.00 | 129.00 | 124.00 | 95.00 | 85.20 |

Continuous Flow SFAAP Soil Columns

Soil Column 6
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | |
| NQ | 127.00 | 107.00 | 121.00 | 139.00 | 6.20 | 128.00 | 120.00 | 144.00 | 128.00 | 131.00 | 108.00 | 108.00 |
| NOQ | 0.00 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 16.00 | 11.00 | 7.50 | 7.20 | 7.00 | 0.00 | 6.10 | 6.80 | 9.80 | 10.00 | 11.10 | 11.20 |
| G | 10.50 | 0.00 | 5.50 | 6.10 | 6.90 | 8.60 | 8.90 | 8.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| NO2-NO3 | 2.30 | 1.20 | 2.70 | 10.30 | 8.30 | 8.60 | 13.10 | 14.00 | 4.00 | 8.00 | 7.40 | 6.00 |
| NH3-N | 4.50 | 0.41 | 4.75 | 3.57 | 6.08 | 3.40 | 2.37 | 3.90 | 3.47 | 2.69 | 4.63 | 5.15 |
| TOC | 362.00 | 54.30 | 350.00 | 364.00 | 289.00 | 322.00 | 329.00 | 334.00 | 358.00 | 367.00 | 743.00 | 767.00 |
| SO4 | 0.00 | 0.00 | 0.00 | 126.00 | 140.00 | 105.00 | 118.00 | 127.00 | 120.00 | 119.00 | 113.00 | 132.00 |

Soil Column 6
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 | 217 | 232 | 245 | 260 | 271 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | | | | | | |
| NQ | 10.80 | 68.00 | 69.00 | 34.00 | 44.00 | 141.00 | 17.60 | 19.30 | 37.50 | 24.80 | 3.20 | 54.00 | 52.30 | 170.00 | 42.80 | 97.00 | 78.00 | 61.90 | 38.50 | 113.00 | 58.84 |
| NOQ | 0.05 | 0.00 | 1.70 | 1.80 | 5.70 | 0.00 | 6.24 | 0.00 | 0.00 | 3.90 | 0.00 | 0.00 | 0.00 | 0.00 | 3.90 | 0.00 | 1.50 | 0.00 | 0.00 | 0.00 | 0.00 |
| CY | 3.70 | 5.60 | 5.70 | 0.00 | 2.20 | 5.90 | 1.20 | 2.00 | 0.00 | 4.30 | 2.30 | 7.60 | 4.70 | 9.10 | 5.00 | 6.90 | 12.50 | 8.00 | 12.00 | 11.20 | 0.00 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NO2-NO3 | 0.90 | 1.10 | 1.20 | 0.00 | 0.60 | 6.50 | 1.20 | 0.70 | 5.80 | 11.60 | 2.80 | 8.70 | 14.90 | 15.80 | 3.00 | 9.00 | 9.00 | 0.00 | 11.00 | 4.20 | 2.40 |
| NH3-N | 1.10 | 0.06 | 1.53 | 0.08 | 8.20 | 4.14 | 10.20 | 12.60 | 10.10 | 5.30 | 7.00 | 0.50 | 0.00 | 3.50 | 4.70 | 8.20 | 10.30 | 9.17 | 9.78 | 0.82 | 0.00 |
| TOC | 362.00 | 273.00 | 286.00 | 106.00 | 217.00 | 50.60 | 50.20 | 46.00 | 49.60 | 0.00 | 13.50 | 27.00 | 0.00 | 38.90 | 25.80 | 26.40 | 47.00 | 21.20 | 68.30 | 15.70 | 9.55 |
| SO4 | | | | | | 68.00 | 0.00 | 41.00 | 0.00 | 0.00 | 0.00 | 57.00 | 0.00 | 123.00 | 124.00 | 93.70 | 260.00 | 46.60 | 62.00 | 60.00 | 16.00 |

# APPENDIX D

# CALCULATION OF Z VALUE

# APPENDIX D

## CALCULATION OF Z VALUE

$$Z\ (0, \_\_\_\_) = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\sigma_1^2/n_1 + \sigma_2^2/n_2}}$$

$$\mu_0 - 1.96\ \sigma_{\bar{x}} \leqq x \leqq \mu_o + 1.96\ \sigma_{\bar{x}}$$

$\bar{x}_1$ = Mean of influent
$\bar{x}_2$ = Mean of effluent
$\sigma_1$ = Standard deviation of influent
$\sigma_2$ = Standard deviation of effluent
n = Sample number
$\mu$ = Mean of population

See Table 4-2.

## Z-TEST VALUES CONTINUOUS FLOW SOIL COLUMNS

| Component | Soil columns 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| Nitroguanidine | -0.37 | 0.17 | 2.42 | -2.34 | -3.95 |
| Nitrosoguanidine | -1.48 | -1.82 | -1.34 | -1.76 | -2.50 |
| Cyanamide | 0.95 | 0.32 | 1.63 | 0.96 | 2.35 |
| Guanidine | 2.05 | 3.35 | 1.40 | 3.66 | 2.18 |
| Nitrate-Nitrate | 0.16 | 0.76 | 1.89 | 0.82 | 1.13 |
| Ammonia nitrogen | 7.18 | 0.46 | -1.48 | 1.85 | -1.30 |
| Sulfate | -0.25 | 0.81 | 6.76 | -0.074 | 3.49 |
| Total Organic Carbon | -3.34 | 1.13 | 2.83 | -0.53 | 5.51 |

Significant = ±1.96

TABLE D-1. PERCENT REDUCTION OF NITROGUANIDINE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 10.40 | 5.90 | -43.27 |
| 2 | 120.07 | 118.56 | - 1.26 |
| 3 | 113.88 | 105.98 | - 6.90 |
| 4 | 112.57 | 77.84 | -30.90 |
| 5 | 120.34 | 85.50 | -29.00 |
| 6 | 112.97 | 62.90 | -44.32 |

TABLE D-2. PERCENT CHANGE OF NITROSOGUANIDINE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 |
| 2 | 0.022 | 0.097 | + 340 |
| 3 | 0.008 | 0.104 | +1,200 |
| 4 | 0.092 | 0.352 | + 282 |
| 5 | 0.017 | 0.244 | +1,335 |
| 6 | 0.058 | 0.915 | +1,478 |

TABLE D-3. PERCENT CHANGE OF GUANIDINE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 0.60 | 0.00 | -100 |
| 2 | 4.45 | 0.424 | - 90 |
| 3 | 6.46 | 0.597 | - 91 |
| 4 | 5.06 | 1.9855 | - 61 |
| 5 | 7.435 | 0 | -100 |
| 6 | 3.76 | 0.51 | - 86 |

TABLE D-4. PERCENT CHANGE OF CYANAMIDE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 0.58 | 0.60 | + 3.44 |
| 2 | 9.465 | 8.252 | -12.8 |
| 3 | 7.255 | 5.1 | -29.7 |
| 4 | 7.81 | 6.1 | -21.9 |
| 5 | 6.316 | 4.127 | -34.7 |
| 6 | 8.265 | 5.324 | -35.6 |

TABLE D-5. PERCENT CHANGE OF AMMONIA-NITROGEN IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 0.25 | 0.48 | +92.00 |
| 2 | 2.413 | 0.084 | -97.00 |
| 3 | 1.089 | 1.0838 | -00.52 |
| 4 | 1.991 | 3.209 | +61.00 |
| 5 | 1.62 | 0.755 | -53.00 |
| 6 | 3.7 | 4.711 | +27.00 |

TABLE D-6. PERCENT CHANGE OF NITRITE-NITRATE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 2.10 | 5.40 | +157.14 |
| 2 | 12.03 | 12.714 | + 5.7 |
| 3 | 8.325 | 6.217 | - 25.3 |
| 4 | 13.33 | 9.1759 | - 31.16 |
| 5 | 5.945 | 5.088 | - 14.4 |
| 6 | 7.32 | 5.503 | - 24.8 |

TABLE D-7. PERCENT CHANGE OF SULFATE IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 0 | 0.89 | |
| 2 | 133.071 | 129.95 | - 2.30 |
| 3 | 120.571 | 103.0 | -14.57 |
| 4 | 118.071 | 37.476 | -68.26 |
| 5 | 81.357 | 77.914 | - 4.2 |
| 6 | 122.57 | 61.5714 | -49.8 |

TABLE D-8. PERCENT CHANGE OF TOC IN SFAAP SOIL

| Column | Influent (mean) | Effluent (mean) | Percent change (mean) |
|---|---|---|---|
| 1 | 2.14 | 7.13 | +233.18 |
| 2 | 11.713 | 14.42 | + 22.8 |
| 3 | 409.01 | 277.154 | - 32.2 |
| 4 | 452.684 | 215.388 | - 52.4 |
| 5 | 313.2 | 340.63 | + 8.8 |
| 6 | 331.611 | 101.127 | - 69.50 |

# APPENDIX E

# PROCEDURE FOR RUNNING LINEAR REGRESSION VIA SAS FOR LABORATORY SOIL COLUMN ANALYSIS

# APPENDIX E

## PROCEDURE FOR RUNNING LINEAR REGRESSION VIA SAS FOR LABORATORY SOIL COLUMN ANALYSIS

### Objective

To conduct data and statistical analysis through the SAS statistical package, particularly the REG linear regression analysis program. The regression is used on the results of soil column experiments. The problem posed is: under laboratory experimental controlled conditions, are there any significant differences between various conditions? This question can be answered by employing regression analysis, analysis of variance, or univariate analysis. The regression analysis is mainly concerned with the relations among variables. There are three major entities in the experiment: namely, influent, effluent, and soil column. The regression analysis is applied to establish the soil column as a "relation" with the theoretical propositions embodied in relation of influent and effluent for different treated columns. Then the test can be conducted to see whether the different treatment of the soil columns has a significant impact on the influent and effluent relation.

### Regression Model

The functional relations are assumed that

EFFLUENT = f (INFLUENT, DAYS)

for each chemical compound in each soil column.

If the functional relation is liner, then the linear regression equation can be represented:

EFFLUENT = intercept + b1*(INFLUENT) + b2*(DAYS) + e

where

EFFLUENT and INFLUENT are the same chemical compound variable collected from the same soil column in effluent and influent sample, respectively.

intercept, b1 and b2, are parameters to be estimated.

e is a random disturbance term.

## Preparing the SAS Data Files

The data files are prepared from 6 data sheets (see Figure 1 for an example). Figure 1 shows one of the six data sheets which contain soil column 6 data information. The influent and effluent chemical compound samples are collected at a point of time (called "DAYS") for each soil column. The variables of interest in this example are NQ, NOQ, CY, G, $NO_2$-$NO_3$, $NH_3N$, TOC, $SO_4$, DAYS and COLUMN (soil column ID). Character "E" or "I" is added as the last digit of the chemical compound variable name to distinguish it as influent variable or effluent variable. The data set is arranged in a rectangular table (see Figure 2). The columns and rows in the table are variables and observations, respectively.

The SAS data file is named as follows:

mmmyy.DAT

where

mmmyy is a meaningful file name identifying the data (up to 8 characters). In this example, MAY86.DAT is used to indicate the date of the data sheet.

Soil Column 6
Influent mg/l

| days | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 |
|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | |
| NH3 | 127.00 | 107.00 | 121.00 | 139.00 | 6.20 | 128.00 | 120.00 | 144.00 |
| NO3 | 0.00 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 | | |
| CY | 16.00 | 11.00 | 7.50 | 7.20 | 7.00 | 0.00 | 6.10 | 6.80 |
| B | 10.50 | 0.00 | 5.50 | 6.10 | 6.90 | 8.60 | | |
| | | | | | | | | |
| | | | | | | | | |
| NO2-NO3 | 2.30 | 1.20 | 2.70 | 10.30 | 8.30 | 8.60 | 13.10 | 14.00 |
| NH2N | 4.50 | 0.41 | 4.75 | 3.57 | 6.08 | 3.40 | 2.37 | 3.90 |
| TOC | 342.00 | 54.30 | 350.00 | 364.00 | 289.00 | 322.00 | 329.00 | 334.00 |
| SO4 | | | | 126.00 | 140.00 | 105.00 | 118.00 | 127.00 |

Soil Column 6
Effluent mg/l

| days | 10 | 38 | 46 | 53 | 61 | 69 | 75 | 83 | 90 | 97 | 104 | 118 | 132 | 176 | 190 | 204 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| test | | | | | | | | | | | | | | | | |
| NH3 | 10.80 | 68.00 | 69.00 | 34.00 | 44.00 | 141.00 | 17.60 | 19.30 | 37.50 | 24.80 | 3.20 | 54.00 | 52.30 | 170.00 | 42.80 | 97.00 |
| NO3 | 0.05 | 0.00 | 1.70 | 1.80 | 5.70 | 0.00 | 6.24 | 0.00 | 0.00 | 3.90 | 0.00 | 0.00 | | | 3.90 | |
| CY | 3.70 | 5.60 | 5.70 | 0.00 | 2.20 | 5.90 | 1.20 | 2.00 | 0.00 | 4.30 | 2.30 | 7.60 | 4.70 | 9.10 | 5.00 | 6.90 |
| B | 7.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| NO2-NO3 | 0.90 | 1.10 | 1.20 | | 0.60 | 6.50 | 1.20 | 0.70 | 5.80 | 11.60 | 2.80 | 8.70 | 14.90 | 15.80 | 3.00 | 9.00 |
| NH2N | 1.10 | 0.06 | 1.53 | 0.08 | 8.20 | 4.14 | 10.20 | 12.60 | 10.10 | 5.30 | 7.00 | 0.50 | 0.00 | 3.50 | 4.70 | 8.20 |
| TOC | 342.00 | 273.00 | 17.00 | 106.00 | 217.00 | 50.60 | 50.20 | 46.00 | 49.60 | | 13.50 | 27.00 | | 38.90 | 470.00 | 26.40 |
| SO4 | | | | | | | 0.00 | 41.00 | 0.00 | | 0.00 | 57.00 | | 123.00 | 124.00 | 93.70 |

Figure 1 Data Sheet of Soil Column 6

```
6 101270   0 1600 1050   230 45036200      108      5  370  740   90  11036200
6 381070  58 1100    0   120   41 5430      680      0  560    0  110    627300
6 461210   0  750  550   270 47535000      690    170  570    0  120  15328600
6 691390   0  720  610  1030 35736400126  1410      0  590    0  650  414 5060 680
6 90  62   0  700  690   830 60828900140   375      0    0    0  580 1010 4960   0
61181280   0    0  860   860 34032200105   560      0  760    0  870   50 2700 570
61761200   0  610    0  1310 23732900118  1700      0  910    0 1580  350 38901230
62041440   0  680    0  1400 39033400127   970      0  690    0  900  820 2640 937
5 101270   0   16  900   190  1635200      127      0  370    0  280   83 2150
5 381160  17 1300    0   150 157 2180     1190     35  550    0  160  168 8120
5 461140   0 1800  930   200 45337100     1250     77  700    0  200  273 1700
5 691520   0  440 2490   860 10051000 32  1480      0  180    0  690  10051000   0
5 90  14   0 1030    0  1230 120 7160 43   776      0 1040    0 1000    050900 360
51181850   0  800  830   510  4032400110   520      0    0    0  442    079300 260
51761300   0    0    0   570 14032400 96   700      0    0    0  530    0323001140
52041340   0    0  920   600 12032700122   820      0  940    0  500    0318001310
2 101330   8 1800 1010   140 220  917       112      2  330    0  240   22 1580
2 38 990  14 1000    0  1810  17 1420       151      0    0    0 1100    0  957
2 461360   0  750    0  1100 466 1180       700     22 1300    0 2570    5 1360
2 691700   0  820  790  1320 290 1100126  1510      0  750    0  860   13 10701260
2 90  67   0  760    0  1440 287 1090120  1490      0  840    0 2460   13 18501350
21181360   0  870  770   990 230 1160242  1272      0  910    0  660    0 20801210
21761300   0  650  910  1500 230  978100  1200      0  860    0 1920    0 11001000
22041450   0  710  970  1600 260 1060127  1410     45  980    0 3000    0 12501270
3 101310   0 3100 1010    30   043800        4      0  240    0  240  140 1640
3 38 950   8 1000    0   150 171 1650      1000     20  550    0  150  193 1400
3 461260   0 2000  970   190 27542900      1130     16  570    0  200  255 1630
3 691520   0    0 1430  2200 11061900114013 90      0    0    0  290   3716800 440
3 90  14   0  730 1430  2560  50636001350   9      0 2010    0 2500   50    0  0
31181264   0    0    0   400  60 861012101120      0    0    0  470   40428001260
31761200   0    0  710   480  5043900105011 00      0  160  420  480  150469001090
32041320   0    0    0   600   03750011601210      0    0    0  600  120455001270
4 101180   0 1200 1060   580 13044900         7      0    0    0 4100   61 1220
4 38 720  17  730    0  1670  14 1250       580     10  290    0  900    9  998
4 461300   0  660 1130  1670 34542400      1230     38  630   39 1250   39 1630
4 691570   0  340 1180  1150 2208950013201400      0  690    0  730   9626200   0
4 90  67   0  680  860  2580  50434001350 109      0  687    0  880   3036400 400
41181300   0  800  830   970 230430001160 410      0  800  830  120   7017100   0
41761200   0  660    0  1470 32041600 8401300      0  710    0 1230  38041200 980
42041400   0  690    0  1500 2704040012701220     20  650 1350  900  55030600 828
```

Figure 2 SAS Data File (MAY96.DAT)

The SAS program file should be named the same way, that is, MAY86.SAS. Other file naming conventions may need to be established for other clients.

Specifying Data Fields

Each data file is a fixed format ASCII data file which looks like Figure 2. The fields are defined as follows:

| | |
|---|---|
| Field 1 (columns 1 - 2) | right justified "COLUMN ID" number |
| Field 2 (columns 2 - 4 | right justified "DAYS" as integer number |
| Field 3 (columns 5 - 8) | right justified "NQI" value with a single digit to the right of an implied decimal point taken from the "NQ" row in influent section of the data sheet. |
| Field 4 (columns 9 - 11) | right justified "NOQI" value with 2 digits to the right of an implied decimal point taken from the "NOQ" row in influent section of the data sheet. |
| Field 5 (columns 12 - 16) | right justified "CYI" value with 2 digits to the right of an implied decimal point taken from the "CY" row in influent section of the data sheet. |
| Field 6 (columns 17 - 21) | right justified "GI" value with 2 digits to the right of an implied decimal point taken from the "G" row in influent section of the data sheet. |
| Field 7 (columns 22 - 26) | right justified "NO2-NO3I" value with 2 digits to the right of an |

| | |
|---|---|
| | implied decimal point taken from the "NO2-NO3" row in influent section of the data sheet. |
| Field 8 (columns 27 - 30) | right justified "NH3NI" value with 2 digits to the right of an implied decimal point taken from the "NH3N" row in influent section of the data sheet. |
| Field 9 (columns 31 - 35) | right justified "TOCI" value with 2 digits to the right of an implied decimal point taken form the "TOC" row in influent section of the data sheet. |
| Field 10 (columns 35 - 38) | right justified "SO4I" value taken from the "SO4" row in influent section of the data sheet. |
| Field 11 (columns 40 - 43) | right justified "NQE" value with a single digit to the right of an implied decimal point taken from the "NQ" row in effluent section of the data sheet. |
| Field 12 (columns 44 - 49) | right justified "NOQE" value with 2 digits to the right of an implied decimal point taken from the "NOQ" row in the effluent section of the data sheet. |
| Field 13 (columns 50 - 54) | right justified "CYE" value with 2 digits to the right of an implied decimal point taken from the "CY" row in effluent section of the data sheet. |
| Field 14 (columns 55 - 59) | right justified "GE" value with 2 digits to the right of an implied decimal point taken from the "G" row in effluent section of the data sheet. |

Field 15 (columns 60 - 64) — right justified "NO2-NO3E" value with 2 digits to the right of an implied decimal point taken from the "NO2-NO3" row in effluent section of the data sheet.

Field 16 (columns 65 - 69) — right justified "NW2NE" value with 2 digits to the right of an implied decimal point taken from the "$NH_3N$" row in effluent section of the data sheet.

Field 17 (columns 70 - 74) — right justified "TOCE" value with 2 digits to the right of an implied decimal point taken from the "TOC" row in effluent section of the data sheet.

Field 18 (columns 75 - 78) — right justified "SO4E" value with a single digit to the right of an implied decimal point taken from the "SO4" row in effluent section of the data sheet.

Some data sheets may contain different format of information. A meaningful variable name should be assigned to each field. The data information for this example has been stored on an SAS external permanent data set with the following field format.

| Variable Name | Field Location | Variable Type |
|---|---|---|
| COLUMN | 1 - 1 | Numeric |
| DAYS | 2 - 4 | Numeric |
| NQI | 5 - 8 | Numeric |
| NOQI | 9 - 11 | Numeric |
| CYI | 12 - 16 | Numeric |
| GI | 17 - 21 | Numeric |
| NO2-NO3I | 22 - 26 | Numeric |
| $NH_3NI$ | 27 - 30 | Numeric |
| TOCI | 31 - 35 | Numeric |
| SO4I | 36 - 38 | Numeric |
| NQE | 40 - 43 | Numeric |
| NOQE | 45 - 49 | Numeric |
| CYE | 50 - 54 | Numeric |
| GE | 55 - 59 | Numeric |

| | | |
|---|---|---|
| NO2-NO3 | 60 - 64 | Numeric |
| NH4E3 | 65 - 69 | Numeric |
| TOCE | 70 - 74 | Numeric |
| SO4E | 75 - 78 | Numeric |

Preparing the SAS Program Files

Each SAS program file is a file containing SAS statement codes. The SAS program file is named as MAY86.SAS.

It is not necessary to run a SAS batch job with an identical data file name and program file name. A SAS program contains data steps and PROCedure steps. The DATA steps prepare SAS data sets and the PROC steps analyze or process SAS data sets.

A complete SAS program job stream is shown in Figure 3. This program contains three blocks. The first two blocks are data-related statements. The third block is procedure-related statements.

The first block of DATA step statements is retrieving an external data file (Figure 2) named "MAY86.DAT". It is shown in Figure 4.

The INFILE statement brings the external data file "MAY86.DAT" into the workspace and creates a SAS data set called PR. The variables SO4I and SO4E from SAS data set. In this case, the SO4 analysis will be performed in another batch run.

The second group of DATA step statements creates 5 subfiles based on variable "COLUMN" (See Figure 5). Each subfile contains the specified soil column information.

The first PROC step statement in Figure 6 is PROC PRINT, which prints out the data content and ensures that the correct data set has been used. The second PROC step statement, PROC REG, executes the regression model.

The regression is run using the MODEL statement. The MODEL statement specify the relation of dependent and independent variables. The block of PROC step statements to perform the regression analysis is shown in Figure 6. This group of PROC step statements can be used repeatedly by changing the date set name.

Making the Runs and Checking the Results

Prepare the appropriate data file and prepare and submit the corresponding run stream as a batch job. Examine the resulting printout as follows, to determine if the results are correct. Refer to Figures 7 and 8, which are part of the results of running the job in Figure 3.

1. Check that the data values were properly entered into the data file. These appear on Figure 7. If not, correct the data file and try again.

2. Check the parameters from regression run. The information of MODEL TOCE = TOCI DAYS for Soil Column 6 appears on Figure 8.

    a. Check the upper corner of right hand side on Figure 8, which prints the "DEP VARIABLE:TOCE" indicating the the dependent variable was specified in the regression

```
/**********************************
/***A COMPLETE SAS PROGRAM *****
/**********************************

DATA PR;
  INFILE MAY36;
  INPUT
    @1    COLUMN    1.
    @2    DAYS      3.
    @5    NQI       4.1
    @9    NOQI      3.2
    @12   CYI       5.2
    @17   GI        5.2
    @22   NO2_NO3I  5.2
    @27   NH3NI     4.2
    @31   TOCI      5.2
    @36   SO4I      3.
    @40   NQE       4.1
    @45   NOQE      5.2
    @50   CYE       5.2
    @55   GE        5.2
    @60   NO2_NO3E  5.2
    @65   NH3NE     5.2
    @70   TOCE      5.2
    @75   SO4E      4.1;
  DROP SO4I SO4E;

DATA C2 C3 C4 C5 C6;
  SET PR;
  IF COLUMN=2 THEN  C2;
  IF COLUMN=3 THEN  C3;
  IF COLUMN=4 THEN  C4;
  IF COLUMN=5 THEN  C5;
  IF COLUMN=6 THEN  C6;


PROC PRINT DATA=C2;

PROC REG DATA=C2;
  MODEL NQE = NQI DAYS;
  MODEL CYE = CYI DAYS;
  MODEL NO2_NO3E = NO2_NO3I DAYS;
  MODEL NH3NE = NH3NI DAYS;
  MODEL TOCE = TOCI DAYS;
```

Figure 3 Actual SAS Program as Batch Job Run Stream

```
PROC PRINT DATA=C3;

PROC REG DATA=C3;
  MODEL NOE = NOI DAYS;
  MODEL OYE = OYI DAYS;
  MODEL NO2_NO3E = NO2_NO3I DAYS;
  MODEL NH3NE = NH3NI DAYS;
  MODEL TOCE = TOCI DAYS;

PROC PRINT DATA=C4;

PROC REG DATA=C4;
  MODEL NOE = NOI DAYS;
  MODEL OYE = OYI DAYS;
  MODEL NO2_NO3E = NO2_NO3I DAYS;
  MODEL NH3NE = NH3NI DAYS;
  MODEL TOCE = TOCI DAYS;

PROC PRINT DATA=C5;

PROC REG DATA=C5;
  MODEL NOE = NOI DAYS;
  MODEL OYE = OYI DAYS;
  MODEL NO2_NO3E = NO2_NO3I DAYS;
  MODEL NH3NE = NH3NI DAYS;
  MODEL TOCE = TOCI DAYS;

PROC PRINT DATA=C6;

PROC REG DATA=C6;
  MODEL NOE = NOI DAYS;
  MODEL OYE = OYI DAYS;
  MODEL NO2_NO3E = NO2_NO3I DAYS;
  MODEL NH3NE = NH3NI DAYS;
  MODEL TOCE = TOCI DAYS;

/************************************
```

Figure 3 Actual SAS Program as Batch Job Run Stream

```
/************************************************
/*** FIRST BLOCK OF DATA STEP STATEMENTS ****
/************************************************

DATA PR;
  INFILE MAY86;
  INPUT
     @1     COLUMN          1.
     @2     DAYS            3.
     @5     NQI             4.1
     @9     NOQI            3.2
     @12    CYI             5.2
     @17    GI              5.2
     @22    NO2_NO3I        5.2
     @27    NH2NI           4.2
     @31    TOCI            5.2
     @36    SO4I            3.
     @40    NQE             4.1
     @45    NOQE            5.2
     @50    CYE             5.2
     @55    GE              5.2
     @60    NO2_NO3E        5.2
     @65    NH2NE           5.2
     @70    TOCE            5.2
     @75    SO4E            4.1;
   DROP SO4I SO4E;

/************************************
```

Figure 4 First Block of DATA Step Statements

```
/************************************************
/*** SECOND BLOCK OF DATA STEP STATEMENTS ***
/************************************************

DATA C2 C3 C4 C5 C6;
  SET PR;
  IF COLUMN=2 THEN C2;
  IF COLUMN=3 THEN C3;
  IF COLUMN=4 THEN C4;
  IF COLUMN=5 THEN C5;
  IF COLUMN=6 THEN C6;

/************************************************
```

Figure 5 Second Block of DATA Step Statements

```
/*************************************
/***THE BLOCK OF PROC STATEMENTS***
/*************************************

PROC PRINT DATA=C2;

PROC REG DATA=C2;
   MODEL NQE = NQI DAYS;
   MODEL CYE = CYI DAYS;
   MODEL NO2_NO3E = NO2_NO3I DAYS;
   MODEL NH3NE = NH3NI DAYS;
   MODEL TOCE = TOCI DAYS;

/*************************************
```

Figure 6 The Block of PROC Statements

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| OBS | COLUMN | DAYS | NGI | NOGI | CYI | GI | NO2_NO3I | $NH_3NI$ | TOCI | NGE | NOGE | CYE | GE | NO2_NO3E | $NH_3NE$ | TOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 10 | 127.0 | 0.00 | 16.0 | 10.5 | 2.3 | 4.50 | 362.0 | 10.8 | 0.05 | 3.7 | 7.4 | 0.9 | 1.10 | 362.0 |
| 2 | 6 | 38 | 107.0 | 0.58 | 11.0 | 0.0 | 1.2 | 0.41 | 54.3 | 68.0 | 0.00 | 5.6 | 0.0 | 1.1 | 0.06 | 273.0 |
| 3 | 6 | 46 | 121.0 | 0.00 | 7.5 | 5.5 | 2.7 | 4.75 | 350.0 | 69.0 | 1.70 | 5.7 | 0.0 | 1.2 | 1.53 | 286.0 |
| 4 | 6 | 69 | 139.0 | 0.00 | 7.2 | 6.1 | 10.3 | 3.57 | 364.0 | 141.0 | 0.00 | 5.9 | 0.0 | 6.5 | 4.14 | 50.6 |
| 5 | 6 | 90 | 6.2 | 0.00 | 7.0 | 6.9 | 8.3 | 6.08 | 289.0 | 37.5 | 0.00 | 0.0 | 0.0 | 5.8 | 10.10 | 49.6 |
| 6 | 6 | 118 | 128.0 | 0.00 | 0.0 | 8.6 | 8.6 | 3.40 | 322.0 | 54.0 | 0.00 | 7.6 | 0.0 | 8.7 | 0.50 | 27.0 |
| 7 | 6 | 176 | 120.0 | 0.00 | 6.1 | 0.0 | 13.1 | 2.37 | 329.0 | 170.0 | 0.00 | 9.1 | 0.0 | 15.8 | 3.50 | 38.9 |
| 8 | 6 | 204 | 144.0 | 0.00 | 6.8 | 0.0 | 14.0 | 3.90 | 334.0 | 97.0 | 0.00 | 6.9 | 0.0 | 9.0 | 8.20 | 26.4 |

Figure 7 Input Data for Soil Column 6

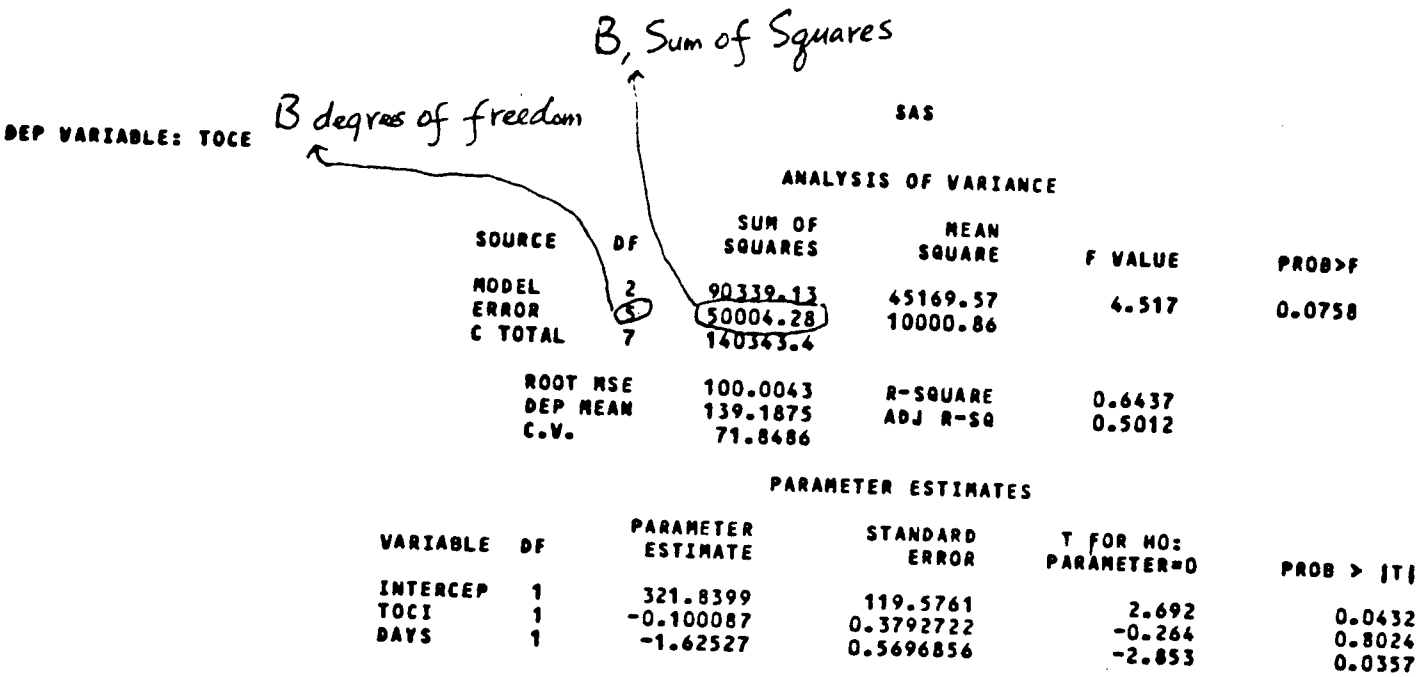


Figure 8 Regression Results of Soil Column 6 for TOC

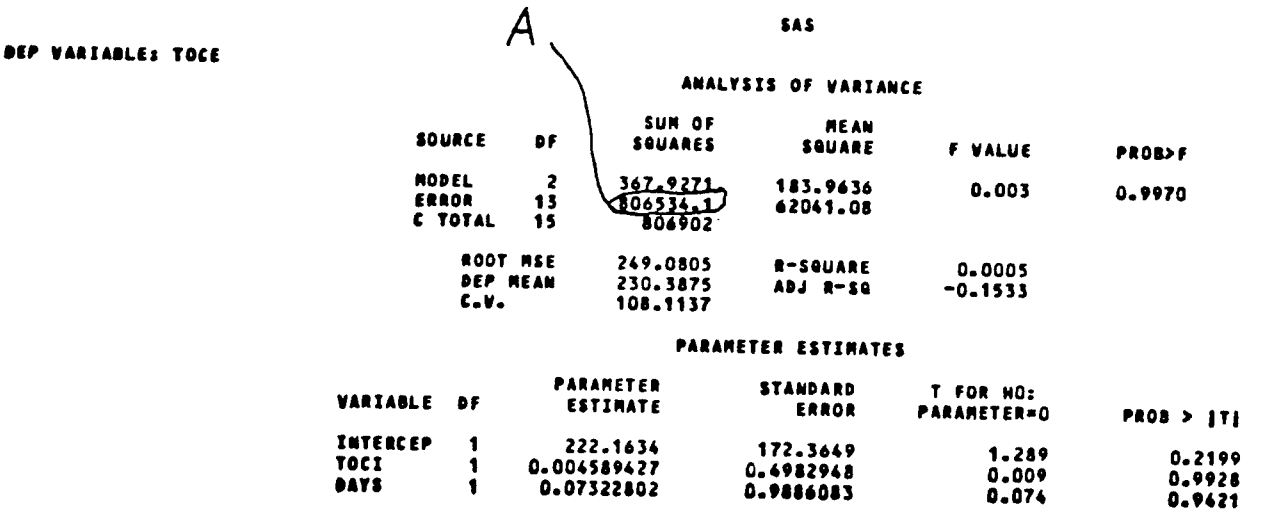


Figure 9 Regression Results of Pooling Soil Column 5 and 6 Together for TOC Model

model. If not, check the MODEL statement to ensure the specification of the model is appropriate.

b. Check the Table of "ANALYSIS OF VARIANCE" on the upper center of Figure 8. "DF" stands for "degrees of freedom". "C TOTAL" degrees of freedom should be (N-1) where N is the total number of observations. In this example, N = 8, and "C TOTAL" equals to 7. If not, check the data file. If there are some missing values in the data file, then the SAS program automatically deletes the observations which contain missing values. An alternative way is to assign "reasonable" values to missing values.

c. "R-SQUARE" in Figure 8 is a measure commonly used to describe how well the regression line fits the observe data. Note the "R-SQUARE" cannot be negative or greater than one, i.e.,

$$0 \leqslant (\text{R-SQUARE}) \leqslant 1$$

A zero value of R-SQUARE indicates the poorest, and a one value the best fit that can be attained.

The increase of the number of independent variables will improve the value of R-SQUARE and give an overly optimistic picture of the performance of the independent variables. "ADJ R-SQ" on Figure 8 stands for "adjusted R-SQUARE" which is defined as:

$$\overline{R^2} = R^2 - ( P - 1 ) \ (1 - R^2) \ / \ ( N - P )$$

where P is the number of independent variables, including intercept term.

The "adjusted R-SQUARE" is a modification of "R-SQUARE" by taking into account the number of independent variables and the number of observations. Note that adjusted R-SQUARE" may be negative; in that case the square root is usually not computed.

d. Check the Table of "PARAMETER ESTIMATES" in the bottom half of Figure 8. The first column of this Table shows the list of independent variables; i.e., INTERCEP, TOCI, and DAYS. The third column is the list of estimated parameters for the corresponding independent variables. The fifth column shows the T-value for the $H_o$ hypothesis that parameter = 0. It becomes a normal rule to set a 5% level of significance, which means that if the deviation of estimated parameter from zero is so great as to occur by chance only 5% of the time, then $H_o$ should be rejected. If the parameters which have values of probability greater than 0.05 in the sixth column, then the null hypothesis of parameter = 0 will be accepted. It implies that this independent variable has no explanatory power in the regression model.

e. Check the sign of the estimated parameters which are in the level of significance. If the sign of any parameter violates the theoretical assumption, then check the data file. If there are some outliers, delete them and try again.

Preparing the Test of Homogeneity of Two Regressions

Appendix F provides a detailed description of the statistical procedure for testing homogeneity of two regressions. Following that procedure, a pair of Soil Columns was picked for the comparison, say Soil Columns 5 and 6 on variable TOC. The homogeneity test sets the null hypothesis that estimated parameters from Soil Columns 5 and 6 for the dependent variable TOCE are the same. From Appendix F, the ratio of (A-B-C)/p to (B+C)/(n+m-2P) will be distributed as F (p, n+M-2P) under the null hypothesis that both groups of observations belong to the same regression model. To perform this test, we need the following sums of squares:

B, sum of square of 8 deviations of dependent variable TOCE from the Soil Column 6 regression estimated by 8 observations with 5 degrees of freedom. B value is shown on the second row of "SUM OF SQUARES" in Table of "ANALYSIS OF VARIANCE" on Figure 8. C can be obtained by picking the counterpart of Soil Column 5 regression run. Pooling the observations of Soil Columns 5 and 6 together, we run the same regression mode again (See Figure 9). The difference between this run and previous runs is that this run uses 16 observations. Sum of square of A can be obtained from Figure 9. The ratio of (A-B-C)/p to (B+C)/(n+m-2p) is F-value. At 5% level of significance, we can test the F-value to see whether to accept the null hypothesis or not.

WESTON

# APPENDIX F

## SOIL COLUMN LINEAR REGRESSION ANALYSIS - PRINT OUT

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DEP VARIABLE: LCYS

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.9732321 | 0.486616 | 19.441 | 0.0024 |
| ERROR | 6 | 0.1501788 | 0.0250298 | | |
| C TOTAL | 8 | 1.123411 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.1582081 | R-SQUARE | 0.8663 |
| DEP MEAN | 1.962953 | ADJ R-SQ | 0.8218 |
| C.V. | 8.0597 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.102105 | 0.6329298 | -0.161 | 0.8771 |
| LCYI | 1 | 0.2432605 | 0.2097824 | 1.160 | 0.2903 |
| LDAYS | 1 | 0.3353723 | 0.05668529 | 5.916 | 0.0010 |

DEP VARIABLE: LTOCE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 12.64171 | 6.320854 | 19.346 | 0.0006 |
| ERROR | 9 | 2.940595 | 0.3267328 | | |
| C TOTAL | 11 | 15.5823 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.5716054 | R-SQUARE | 0.8113 |
| DEP MEAN | 3.958613 | ADJ R-SQ | 0.7693 |
| C.V. | 14.43954 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 10.90434 | 1.548457 | 7.042 | 0.0001 |
| LTOCI | 1 | -0.488977 | 0.2952621 | -1.656 | 0.1321 |
| LDAYS | 1 | -0.881522 | 0.1906459 | -4.624 | 0.0012 |

DEP VARIABLE: LN_NH3E

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 8.829262 | 4.414631 | 1.805 | 0.2192 |
| ERROR | 9 | 22.01452 | 2.446058 | | |
| C TOTAL | 11 | 30.84378 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 1.563988 | R-SQUARE | 0.2863 |
| DEP MEAN | 0.5660447 | ADJ R-SQ | 0.1276 |
| C.V. | 276.3011 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -2.22412 | 2.251027 | -0.988 | 0.3489 |
| LN_NH3I | 1 | 1.101066 | 0.6827012 | 1.613 | 0.1412 |
| LDAYS | 1 | 0.3213693 | 0.4755084 | 0.676 | 0.5161 |

DEP VARIABLE: LNO2_3E

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 11.75263 | 5.876314 | 7.775 | 0.0109 |
| ERROR | 9 | 6.802492 | 0.7558324 | | |
| C TOTAL | 11 | 18.55512 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.8693862 | R-SQUARE | 0.6334 |
| DEP MEAN | 1.168453 | ADJ R-SQ | 0.5519 |
| C.V. | 74.40456 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.106066 | 1.252553 | -0.085 | 0.9344 |
| LNO2_3I | 1 | 1.611849 | 0.4527157 | 3.560 | 0.0061 |
| LDAYS | 1 | -0.334373 | 0.3384619 | -0.988 | 0.3490 |

DEP VARIABLE: LNOE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 2.309424 | 1.154712 | 2.630 | 0.1325 |
| ERROR | 8 | 3.512595 | 0.4390743 | | |
| C TOTAL | 10 | 5.822019 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.6626268 | R-SQUARE | 0.3967 |
| DEP MEAN | 4.146302 | ADJ R-SQ | 0.2458 |
| C.V. | 15.98115 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.352927 | 1.403131 | 0.964 | 0.3632 |
| LNQI | 1 | 0.1665854 | 0.2304671 | 0.723 | 0.4904 |
| LDAYS | 1 | 0.4452449 | 0.2070315 | 2.151 | 0.0637 |

| OBS | COLUMN | DAYS | LDAYC | LNI | LNOI | LCYI | LGI | LNO2_3I | LN_NH3I | LTOCI | LNQE | LNOQE | LCYE | LGE | LNO2_3E | LN_NH3E | LTOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 10 | 3.30259 | 4.64419 | . | 2.77259 | 2.35138 | 0.83291 | 1.50408 | 5.89164 | 2.37955 | -2.9957 | 1.30833 | 2.00148 | -0.1054 | 0.0953 | 5.89164 |
| 2 | 6 | 38 | 3.63759 | 4.67283 | -0.54473 | 2.39790 | . | 0.18232 | -0.39160 | 3.99452 | 4.21951 | . | 1.72277 | . | 0.0953 | -2.8134 | 5.60947 |
| 3 | 6 | 46 | 3.82864 | 4.72579 | . | 2.01490 | 1.70475 | 0.99325 | 1.55814 | 5.85793 | 4.23411 | 0.5306 | 1.74047 | . | 0.1823 | 0.4253 | 5.65599 |
| 4 | 6 | 64 | 4.25411 | 4.73447 | . | 1.97408 | 1.90829 | 2.33214 | 1.27257 | 5.89715 | 4.94876 | . | 1.77495 | . | 1.8718 | 1.4207 | 3.92395 |
| 5 | 6 | 90 | 4.49981 | 1.92455 | . | 1.94591 | 1.93152 | 2.11626 | 1.80500 | 5.66643 | 3.62434 | . | . | . | 1.7579 | 2.3125 | 3.90399 |
| 6 | 6 | 119 | 4.77968 | 4.65203 | . | . | 2.15176 | 2.15176 | 1.22378 | 5.77455 | 3.98898 | . | 2.02815 | . | 2.1633 | -0.6931 | 3.29584 |
| 7 | 6 | 176 | 5.17048 | 4.78749 | . | 1.80829 | . | 2.57261 | 0.86289 | 5.79606 | 5.13580 | . | 2.20827 | . | 2.7600 | 1.2528 | 3.66099 |
| 8 | 6 | 204 | 5.31712 | 4.96981 | . | 1.91692 | . | 2.63906 | 1.36098 | 5.81114 | 4.57471 | . | 1.93152 | . | 2.1972 | 2.1041 | 3.27336 |
| 9 | 6 | 232 | 5.44674 | 4.85203 | . | 2.28238 | . | 1.38629 | 1.24415 | 5.83053 | 4.12552 | . | 2.07944 | . | -1.6094 | 2.2159 | 3.05400 |
| 10 | 6 | 245 | 5.50126 | 4.87520 | . | 2.30259 | . | 2.07944 | 0.98954 | 5.90536 | 3.65066 | . | 2.48491 | . | 2.3979 | 2.2803 | 4.22391 |
| 11 | 6 | 260 | 5.56068 | 4.69213 | . | 2.40695 | . | 2.00148 | 1.53256 | 6.61070 | 4.72739 | . | 2.41591 | . | 1.4351 | -0.1985 | 2.75366 |
| 12 | 6 | 271 | 5.60717 | 4.69213 | . | 2.41591 | . | 1.79176 | 1.63900 | 6.64249 | . | . | . | . | 0.8755 | -1.6094 | 2.25654 |
| 13 | 6 | 317 | 5.37990 | . | . | . | . | . | . | . | 4.35671 | 0.4055 | 2.52573 | . | 2.1972 | 2.3321 | 3.85015 |

DEP VARIABLE: LN_NH3I

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 1.352808 | 0.6764039 | 39.972 | 0.0008 |
| ERROR | 5 | 0.08461062 | 0.01692212 | | |
| C TOTAL | 7 | 1.437418 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.1300351 | R-SQUARE | 0.9411 |
| DEP MEAN | 0.2733646 | ADJ R-SQ | 0.9176 |
| C.V. | 47.58665 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.08785 | 0.2106021 | 5.165 | 0.0036 |
| LN_NH3I | 1 | 0.4658188 | 0.05210571 | 8.940 | 0.0003 |
| LDAYS | 1 | -0.211404 | 0.04721446 | -4.478 | 0.0065 |

DEP VARIABLE: LCYF

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.3977077 | 0.1988538 | 0.416 | 0.6928 |
| ERROR | 3 | 1.43466 | 0.47822 | | |
| C TOTAL | 5 | 1.832368 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.6915345 | R-SQUARE | 0.2170 |
| DEP MEAN | 1.616145 | ADJ R-SQ | -0.3049 |
| C.V. | 42.78913 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.711493 | 1.485009 | 1.153 | 0.3326 |
| LCYI | 1 | 0.2004886 | 0.2660636 | 0.754 | 0.5059 |
| LDAYS | 1 | -0.108952 | 0.4421112 | -0.246 | 0.3212 |

DEP VARIABLE: LTOCC

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 11.21996 | 5.60998 | 6.960 | 0.0149 |
| ERROR | 9 | 7.254427 | 0.8060474 | | |
| C TOTAL | 11 | 13.47439 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.8978014 | R-SQUARE | 0.6073 |
| DEP MEAN | 5.44166 | ADJ R-SQ | 0.5201 |
| C.V. | 16.49867 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.280821 | 1.739495 | 0.736 | 0.4803 |
| LTOCI | 1 | -0.116789 | 0.2964226 | -0.394 | 0.7028 |
| LDAYS | 1 | 1.034471 | 0.2870248 | 3.604 | 0.0057 |

DEP VARIABLE: LNO2_3I

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 2.91749 | 1.408745 | 65.537 | 0.0001 |
| ERROR | 9 | 0.1934575 | 0.02149528 | | |
| C TOTAL | 11 | 3.010947 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.1466127 | R-SQUARE | 0.9357 |
| DEP MEAN | 1.477515 | ADJ R-SQ | 0.9215 |
| C.V. | 9.922924 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 0.6737605 | 0.2140813 | 3.147 | 0.0118 |
| LNO2_3I | 1 | 0.7988282 | 0.0836481 | 9.550 | 0.0001 |
| LDAYS | 1 | -0.107534 | 0.05940263 | -1.810 | 0.1037 |

DEP VARIABLE: LNQF

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.962659 | 0.4813295 | 1.211 | 0.3421 |
| ERROR | 9 | 3.576596 | 0.3973996 | | |
| C TOTAL | 11 | 4.539255 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.6303964 | R-SQUARE | 0.2121 |
| DEP MEAN | 4.305153 | ADJ R-SQ | 0.0370 |
| C.V. | 14.64283 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 3.089899 | 1.079897 | 2.861 | 0.0187 |
| LNQI | 1 | -0.0320304 | 0.1446563 | -0.221 | 0.8297 |
| LDAYS | 1 | 0.291897 | 0.1883403 | 1.550 | 0.1556 |

| OBS | COLUMN | DAYS | LDAYS | LNQI | LNOQI | LCYI | LGI | LNO2_3I | LN_NH3I | LTOCI | LNQE | LNOQE | LCYE | LGE | LNO2_3E | LN_NH3E | LTOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 10 | 2.30259 | 4.84419 | . | -1.8326 | 2.19722 | 0.64185 | -1.8326 | 5.86363 | 2.54160 | . | 1.30833 | . | 1.02962 | -0.18633 | 3.06805 |
| 2 | 5 | 31 | 3.43399 | 4.75359 | -1.772 | 2.5649 | . | 0.40547 | 0.4511 | 3.08191 | 4.77912 | -1.0498 | 1.70475 | . | 0.47000 | 0.51879 | 4.39692 |
| 3 | 5 | 48 | 3.87120 | 4.73620 | . | 2.8904 | 2.23001 | 0.69315 | 1.5107 | 5.91620 | 4.82831 | -0.2614 | 1.94591 | . | 0.69315 | 1.00430 | 2.83321 |
| 4 | 5 | 69 | 4.23411 | 5.02388 | . | 1.4816 | 3.21487 | 2.15176 | 0.0000 | 6.23441 | 4.99721 | . | 0.58779 | . | 1.93152 | 0.00000 | 6.23441 |
| 5 | 5 | 90 | 4.49981 | 0.33647 | . | 2.3321 | . | 2.50960 | 0.1823 | 4.27110 | 4.35157 | . | 2.34181 | . | 2.30259 | . | 6.23245 |
| 6 | 5 | 110 | 4.70048 | 5.22036 | . | 2.0794 | 2.11626 | 1.62924 | -0.9163 | 5.78074 | 3.95124 | . | . | . | 1.48614 | . | 6.67582 |
| 7 | 5 | 124 | 4.82028 | 4.86753 | . | . | . | 1.74047 | 0.3365 | 5.78074 | 4.24850 | . | . | . | 1.66771 | . | 5.77765 |
| 8 | 5 | 204 | 5.31812 | 4.89784 | . | . | 2.21920 | 1.79176 | 0.1823 | 5.78996 | 4.40672 | . | 2.24071 | . | 1.60944 | . | 5.76205 |
| 9 | 5 | 232 | 5.44674 | 4.86753 | . | . | 1.82455 | 2.70805 | 1.7596 | 5.79301 | 4.21361 | . | 1.45862 | . | 1.94591 | 0.85015 | 5.80513 |
| 10 | 5 | 245 | 5.50126 | 4.84419 | . | 0.6931 | 2.00148 | 1.79176 | 0.0000 | 5.84354 | 4.19570 | . | . | . | 1.60944 | 0.00000 | 5.78690 |
| 11 | 5 | 260 | 5.56068 | 4.70048 | . | . | 1.74047 | 1.74047 | 0.0000 | 6.50578 | 4.84419 | . | . | . | 1.52606 | 0.00000 | 6.34212 |
| 12 | 5 | 271 | 5.60212 | 4.79579 | . | 2.8094 | 2.07944 | 1.79176 | 0.5128 | 6.51471 | 4.30407 | . | 1.80829 | . | 1.45862 | 0.00000 | 6.38519 |
| 13 | 5 | 287 | 5.37990 | . | . | . | . | . | . | . | 4.35671 | -2.9957 | . | . | 1.79176 | 0.00000 | 5.75574 |

DEP VARIABLE: LCYE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.9251239 | 0.4625619 | 4.207 | 0.0564 |
| ERROR | 8 | 0.8796864 | 0.1099608 | | |
| C TOTAL | 10 | 1.80431 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.3316034 | R-SQUARE | 0.5126 |
| DEP MEAN | 2.002883 | ADJ R-SQ | 0.3907 |
| C.V. | 16.55626 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.0031802 | 0.7647154 | -0.004 | 0.9968 |
| LCYI | 1 | -0.0701317 | 0.4060174 | -0.173 | 0.8672 |
| LDAYS | 1 | 0.4410143 | 0.1844107 | 2.391 | 0.0438 |

DEP VARIABLE: LTOCE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 22.44178 | 11.22089 | 15.926 | 0.0011 |
| ERROR | 9 | 6.341173 | 0.7045748 | | |
| C TOTAL | 11 | 28.78296 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.8393895 | R-SQUARE | 0.7797 |
| DEP MEAN | 4.985771 | ADJ R-SQ | 0.7307 |
| C.V. | 16.8357 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -3.24578 | 1.55324 | -2.090 | 0.0662 |
| LTOCI | 1 | 0.5206339 | 0.2415086 | 2.156 | 0.0595 |
| LDAYS | 1 | 1.104364 | 0.2674031 | 4.130 | 0.0026 |

DEP VARIABLE: LN_NHZE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 17.4995 | 8.74975 | 9.376 | 0.0063 |
| ERROR | 9 | 8.39917 | 0.9332411 | | |
| C TOTAL | 11 | 25.89867 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.9660441 | R-SQUARE | 0.6757 |
| DEP MEAN | 0.3496526 | ADJ R-SQ | 0.6036 |
| C.V. | 276.2868 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -3.73584 | 1.432193 | -2.608 | 0.0283 |
| LN_NHZI | 1 | 0.7650074 | 0.3467718 | 2.206 | 0.0548 |
| LDAYS | 1 | 0.8021694 | 0.3134901 | 2.559 | 0.0307 |

DEP VARIABLE: LN02_3I

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 2.423036 | 1.211518 | 2.245 | 0.1618 |
| ERROR | 9 | 4.855867 | 0.5395407 | | |
| C TOTAL | 11 | 7.278902 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.7345344 | R-SQUARE | 0.3329 |
| DEP MEAN | 2.062079 | ADJ R-SQ | 0.1846 |
| C.V. | 35.62106 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 4.139922 | 1.65306 | 2.504 | 0.0336 |
| LN02_3I | 1 | 0.03455499 | 0.5298068 | 0.065 | 0.9494 |
| LDAYS | 1 | -0.464437 | 0.2191903 | -2.119 | 0.0632 |

DEP VARIABLE: LNSE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 13.5431 | 6.77155 | 4.785 | 0.0384 |
| ERROR | 9 | 12.73661 | 1.415179 | | |
| C TOTAL | 11 | 26.27971 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 1.189613 | R-SQUARE | 0.5153 |
| DEP MEAN | 3.878537 | ADJ R-SQ | 0.4076 |
| C.V. | 30.67169 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -2.78925 | 2.415168 | -1.155 | 0.2779 |
| LNQI | 1 | 0.4575391 | 0.4196834 | 1.090 | 0.3040 |
| LDAYS | 1 | 0.9832635 | 0.3572785 | 2.752 | 0.0224 |

| OBS | COLUM | DAYS | LDAYS | LNI | LNOI | LCYI | LGI | LNO2_3I | LN_NH3I | LTOCI | LNQE | LNOQE | LCYE | LGE | LNO2_3E | LN_NH3E | LTOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 10 | 2.30259 | 4.77068 | . | 2.49491 | 2.36085 | 1.75786 | 0.2624 | 6.10702 | -0.35667 | . | . | . | 3.71357 | -0.4943 | 2.50144 |
| 2 | 4 | 31 | 3.43399 | 4.27667 | -1.7720 | 1.98787 | . | 2.81541 | -1.9661 | 2.52573 | 4.36044 | -2.3026 | 1.06471 | . | 2.19722 | -2.4079 | 2.30058 |
| 3 | 4 | 46 | 3.82864 | 4.36753 | . | 1.68707 | 2.42480 | 2.81541 | 1.2384 | 6.04973 | 4.81218 | -0.9676 | 1.84055 | -0.94161 | 2.52573 | -0.9416 | 2.79117 |
| 4 | 4 | 67 | 4.20411 | 5.05625 | . | 1.22378 | 2.46810 | 2.44235 | 0.7885 | 6.79682 | 4.94164 | . | 1.93152 | . | 1.98787 | -0.0408 | 5.56834 |
| 5 | 4 | 90 | 4.49981 | 1.90211 | . | 1.91692 | 2.15176 | 3.25037 | -0.6931 | 6.07304 | 2.33876 | . | 1.92716 | . | 2.17475 | -1.2040 | 5.89715 |
| 6 | 4 | 111 | 4.77068 | 4.36753 | . | 2.07944 | 2.11626 | 2.27213 | 0.8329 | 6.06379 | 3.71357 | . | 2.07944 | 2.11626 | 0.18232 | -0.3567 | 5.14166 |
| 7 | 4 | 175 | 5.16478 | 4.78749 | . | 1.68707 | . | 2.68785 | 1.1632 | 6.03069 | 4.86753 | . | 1.96009 | . | 2.50960 | 1.3350 | 6.02102 |
| 8 | 4 | 204 | 5.31812 | 4.94164 | . | 1.93152 | . | 2.70805 | 0.9933 | 6.00141 | 4.80402 | -1.6094 | 1.87180 | 2.60269 | 2.19722 | 1.7047 | 5.72359 |
| 9 | 4 | 232 | 5.44674 | 4.90527 | -1.6094 | 2.30259 | . | 2.30259 | 0.8109 | 6.05209 | 3.36038 | . | 2.11626 | . | 2.19722 | 2.9285 | 4.29729 |
| 10 | 4 | 245 | 5.50136 | 4.29035 | . | 2.34181 | . | 2.19722 | 0.6098 | 6.03548 | 4.98361 | . | 2.88480 | 1.74047 | 1.94591 | 0.5365 | 6.31355 |
| 11 | 4 | 260 | 5.56068 | 4.80402 | . | 2.33214 | . | 2.09186 | 0.7655 | 6.75460 | 4.43438 | -0.3567 | 2.25129 | . | 1.50408 | 1.1282 | 6.84375 |
| 12 | 4 | 271 | 5.60312 | 4.73620 | 0.2624 | 2.33214 | . | 2.04122 | 0.6931 | 6.72022 | 4.53260 | -0.5108 | 2.10413 | . | 1.60944 | 2.0082 | 6.42972 |
| 13 | 4 | 217 | 5.37799 | . | . | . | . | . | . | . | 3.76120 | 1.2238 | 1.87180 | . | 1.38629 | 2.6741 | 4.54966 |

DEP VARIABLE: LN_NH3I

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 2.423449 | 1.211725 | 3.615 | 0.0835 |
| ERROR | 7 | 2.346299 | 0.3351856 | | |
| C TOTAL | 9 | 4.769749 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.5789522 | R-SQUARE | 0.5081 |
| DEP MEAN | 0.05093489 | ADJ R-SQ | 0.3675 |
| C.V. | 1136.652 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.579421 | 1.238076 | -0.450 | 0.6664 |
| LN_NH3I | 1 | 0.7332258 | 0.2726864 | 2.689 | 0.0311 |
| LDAYS | 1 | 0.1117735 | 0.2630362 | 0.425 | 0.6836 |

DEP VARIABLE: LCYE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 1.309611 | 0.6543054 | 1.262 | 0.4421 |
| ERROR | 2 | 1.036867 | 0.5184336 | | |
| C TOTAL | 4 | 2.345478 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.7200234 | R-SQUARE | 0.5579 |
| DEP MEAN | 1.875105 | ADJ R-SQ | 0.1159 |
| C.V. | 38.39909 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -2.11653 | 5.722973 | -0.370 | 0.7470 |
| LCYI | 1 | 0.3950508 | 1.038147 | 0.381 | 0.7402 |
| LDAYS | 1 | 0.7769985 | 0.8577343 | 0.906 | 0.4606 |

DEP VARIABLE: LTOCE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 22.03704 | 11.01852 | 22.364 | 0.0005 |
| ERROR | 8 | 3.941599 | 0.4926999 | | |
| C TOTAL | 10 | 25.97863 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.7019258 | R-SQUARE | 0.8483 |
| DEP MEAN | 5.181642 | ADJ R-SQ | 0.8103 |
| C.V. | 13.5464 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -2.23703 | 1.296189 | -1.726 | 0.1227 |
| LTOCI | 1 | 0.240844 | 0.2036706 | 1.183 | 0.2710 |
| LDAYS | 1 | 1.291148 | 0.2228195 | 5.795 | 0.0004 |

DEP VARIABLE: LNO2_3E

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 1.672492 | 0.8362458 | 8.819 | 0.0095 |
| ERROR | 8 | 0.7585447 | 0.09481809 | | |
| C TOTAL | 10 | 2.431036 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.3079255 | R-SQUARE | 0.6880 |
| DEP MEAN | 1.323143 | ADJ R-SQ | 0.6100 |
| C.V. | 23.27228 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.735212 | 0.5556477 | -1.323 | 0.2223 |
| LNO2_3I | 1 | -0.113736 | 0.1322882 | -0.860 | 0.4149 |
| LDAYS | 1 | 0.4760695 | 0.1463963 | 3.252 | 0.0117 |

DEP VARIABLE: LNQC

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 16.76566 | 8.382828 | 5.467 | 0.0319 |
| ERROR | 8 | 12.26748 | 1.533435 | | |
| C TOTAL | 10 | 29.03314 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 1.23332 | R-SQUARE | 0.5775 |
| DEP MEAN | 4.215108 | ADJ R-SQ | 0.4718 |
| C.V. | 29.37312 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 11.43674 | 16.89414 | 0.680 | 0.5157 |
| LNQI | 1 | -2.68949 | 3.522045 | -0.764 | 0.4670 |
| LDAYS | 1 | 1.227573 | 0.3731635 | 3.290 | 0.0110 |

| OBS | COLUMN | DAYS | LDAYS | LMCI | LNQI | LCYI | LGI | LNQ2_3I | LN_NH3I | LTOCI | LNQE | LNQE | LCYE | LGE | LNQ2_3E | LN_NH3E | LTOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 10 | 2.30259 | 4.37520 | . | 3.43399 | 2.31254 | -1.2040 | . | 6.08222 | -0.91629 | . | 0.87547 | . | 0.87547 | 0.33647 | 2.79728 |
| 2 | 3 | 29 | 3.43399 | 4.55338 | -2.5257 | 2.30259 | . | 0.4055 | 0.53649 | 2.80336 | 4.60517 | -1.6094 | 1.70475 | . | 0.40547 | 0.65752 | 2.63906 |
| 3 | 3 | 46 | 3.82864 | 4.83628 | . | 2.99573 | 2.27213 | 0.6419 | 1.01160 | 6.06146 | 4.72739 | -1.8326 | 1.74047 | . | 0.69315 | 0.93609 | 2.79117 |
| 4 | 3 | 60 | 4.09434 | 5.02388 | . | . | 2.66026 | 3.0910 | 0.09531 | 6.42811 | 4.93447 | . | . | . | 1.06471 | -0.99425 | 5.12396 |
| 5 | 3 | 98 | 4.58041 | 0.33647 | . | 1.98787 | 2.66026 | 3.2426 | -0.69315 | 6.45520 | -0.10536 | . | 3.00072 | . | 3.21888 | -0.69315 | . |
| 6 | 3 | 119 | 4.77068 | 4.83949 | . | . | . | 1.3863 | -0.51083 | 4.45551 | 4.71850 | . | . | . | 1.54756 | -0.91629 | 6.05912 |
| 7 | 3 | 176 | 5.17048 | 4.74749 | . | . | 1.96009 | 1.5686 | -0.69315 | 6.08450 | 4.70048 | . | 0.47000 | 1.43503 | 1.56862 | 0.40547 | 6.15060 |
| 8 | 3 | 204 | 5.31812 | 4.36250 | . | . | . | 1.7918 | . | 5.92693 | 4.79579 | . | . | . | 1.79176 | 0.18232 | 6.12030 |
| 9 | 3 | 232 | 5.44674 | 4.88280 | . | . | 1.84055 | 2.7081 | 1.49515 | 6.00389 | 4.69135 | . | 1.64866 | . | 1.38629 | 1.00063 | 6.12905 |
| 10 | 3 | 245 | 5.50136 | 4.87520 | . | 0.91629 | 1.75786 | 1.9459 | 0.00000 | 6.02587 | 4.71850 | . | 2.05412 | . | 1.94591 | 0.00000 | 6.06843 |
| 11 | 3 | 260 | 5.56068 | 4.80402 | . | 0.69315 | 1.80829 | 1.9021 | 0.00000 | 6.77765 | 4.66344 | . | . | . | 1.68640 | 0.00000 | 6.70073 |
| 12 | 3 | 271 | 5.60313 | 4.82831 | . | 1.38629 | . | 1.7228 | 0.00000 | 6.77422 | 4.72739 | . | . | . | 1.58924 | 0.11333 | 6.41836 |
| 13 | 3 | 317 | 5.77990 | . | . | . | . | . | . | . | 4.88280 | . | 2.30259 | . | 2.07944 | 0.00000 | 5.37990 |

DEP VARIABLE: LN_NH3E

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.7392103 | 0.3696051 | 1.611 | 0.2884 |
| ERROR | 5 | 1.147049 | 0.2294099 | | |
| C TOTAL | 7 | 1.83626 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.4789675 | R-SQUARE | 0.3919 |
| DEP MEAN | -1.98429 | ADJ R-SQ | 0.1486 |
| C.V. | -24.1379 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | -0.793864 | 1.055633 | -0.752 | 0.4859 |
| LN_NH3I | 1 | -0.955965 | 0.5363353 | -1.782 | 0.1348 |
| LDAYS | 1 | -0.0709044 | 0.1662288 | -0.427 | 0.6875 |

DEP VARIABLE: LTOCE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 0.1027668 | 0.05138339 | 0.984 | 0.4147 |
| ERROR | 8 | 0.4175412 | 0.05219265 | | |
| C TOTAL | 10 | 0.5203079 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.2284571 | R-SQUARE | 0.1975 |
| DEP MEAN | 2.663543 | ADJ R-SQ | -0.0031 |
| C.V. | 8.577187 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.80764 | 0.7875397 | 2.295 | 0.0508 |
| LTOCI | 1 | 0.4874522 | 0.3567766 | 1.366 | 0.2090 |
| LDAYS | 1 | -0.0718128 | 0.08027531 | -0.895 | 0.3971 |

DEP VARIABLE: LNO2_3E

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 4.311659 | 2.15583 | 10.479 | 0.0058 |
| ERROR | 8 | 1.645906 | 0.2057382 | | |
| C TOTAL | 10 | 5.957565 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.4535837 | R-SQUARE | 0.7237 |
| DEP MEAN | 2.355627 | ADJ R-SQ | 0.6547 |
| C.V. | 19.25533 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.319425 | 0.6923689 | 1.906 | 0.0932 |
| LNO2_3I | 1 | 1.390923 | 0.3290901 | 4.227 | 0.0029 |
| LDAYS | 1 | -0.453219 | 0.2193867 | -2.066 | 0.0727 |

DEP VARIABLE: LCYE

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 1.043979 | 0.5219895 | 8.333 | 0.0111 |
| ERROR | 8 | 0.5011291 | 0.06264114 | | |
| C TOTAL | 10 | 1.545108 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.2502821 | R-SQUARE | 0.6757 |
| DEP MEAN | 2.228845 | ADJ R-SQ | 0.5946 |
| C.V. | 11.22923 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 1.509991 | 0.9409217 | 1.605 | 0.1472 |
| LCYI | 1 | -0.27553 | 0.3075387 | -0.896 | 0.3965 |
| LDAYS | 1 | 0.2803225 | 0.08709767 | 3.218 | 0.0123 |

DEP VARIABLE: LMOS

ANALYSIS OF VARIANCE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PROB>F |
|---|---|---|---|---|---|
| MODEL | 2 | 4.584402 | 2.292201 | 13.217 | 0.0029 |
| ERROR | 8 | 1.387471 | 0.1734339 | | |
| C TOTAL | 10 | 5.971874 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.416454 | R-SQUARE | 0.7677 |
| DEP MEAN | 4.629619 | ADJ R-SQ | 0.7096 |
| C.V. | 8.995426 | | |

PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > \|T\| |
|---|---|---|---|---|---|
| INTERCEP | 1 | 2.238572 | 0.9057852 | 2.471 | 0.0386 |
| LNQI | 1 | -0.163988 | 0.145159 | -1.130 | 0.2913 |
| LDAYS | 1 | 0.663067 | 0.1311384 | 5.056 | 0.0010 |

| OBS | COLUMN | DAYS | LDAYS | LNQI | LNOQI | LCYI | LGI | LNO2_3I | LN_NH3I | LTOCI | LNQE | LNOQE | LCYE | LGE | LNO2_3E | LN_NH3E | LTOCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 10 | 2.30259 | 4.89035 | -2.5257 | 2.39037 | 2.31254 | 0.33647 | 0.7885 | 2.21594 | 2.41591 | -3.9120 | 1.19392 | . | 0.87547 | -1.5141 | 2.76001 |
| 2 | 2 | 38 | 3.63759 | 4.59512 | -1.9661 | 2.30259 | . | 2.39591 | -1.7720 | 2.65324 | 2.71469 | . | . | . | 2.39790 | . | 2.25863 |
| 3 | 2 | 46 | 3.82864 | 4.91265 | . | 2.01490 | . | 2.39790 | 1.5390 | 2.46810 | 4.24850 | -1.5141 | 2.56495 | . | 3.24669 | -2.9957 | 2.61007 |
| 4 | 2 | 69 | 4.23411 | 5.13580 | . | 2.10413 | 2.06686 | 2.58022 | 1.0647 | 2.39790 | 5.01728 | . | 2.01490 | . | 2.15176 | -2.0402 | 2.37024 |
| 5 | 2 | 90 | 4.49981 | 1.90211 | . | 2.02815 | . | 2.66723 | 1.0543 | 2.38876 | 5.00395 | . | 2.12823 | . | 3.20275 | -2.0402 | 2.91777 |
| 6 | 2 | 116 | 4.77068 | 4.91265 | . | 2.16332 | 2.04122 | 2.29253 | 0.8329 | 2.45101 | 4.84576 | . | 2.20827 | . | 1.88707 | . | 3.03495 |
| 7 | 2 | 176 | 5.17048 | 4.86753 | . | 1.87180 | 2.20827 | 2.70805 | 0.8329 | 2.28034 | 4.78749 | . | 2.15176 | . | 2.95491 | . | 2.39790 |
| 8 | 2 | 204 | 5.31812 | 4.97673 | . | 1.96009 | 2.27213 | 2.77259 | 0.9555 | 2.36085 | 4.94876 | -0.7985 | 2.28238 | . | 3.40120 | . | 2.52573 |
| 9 | 2 | 232 | 5.44674 | 4.85981 | . | 2.30259 | . | 2.39790 | 0.2624 | 2.65324 | 4.84419 | . | 2.52573 | . | 2.39790 | -2.0402 | 2.63906 |
| 10 | 2 | 245 | 5.50126 | 4.89035 | . | 2.37955 | . | 2.39790 | 0.8502 | 2.29455 | 4.85203 | . | 2.58022 | . | 1.94591 | -1.4271 | 2.38876 |
| 11 | 2 | 260 | 5.56068 | 4.72739 | . | 2.39790 | . | 2.33214 | 0.7324 | 2.45959 | 4.73620 | . | 2.41591 | . | 1.93152 | -1.5141 | 2.81541 |
| 12 | 2 | 271 | 5.60212 | 4.74493 | . | 2.33214 | . | 2.33214 | 0.9282 | 3.03975 | 5.22575 | . | 2.45101 | . | 1.91692 | -2.3026 | 2.83908 |
| 13 | 2 | 217 | 5.37990 | . | . | . | . | . | . | . | 4.91998 | -1.2040 | 2.30259 | . | 2.19722 | -2.3026 | 2.80336 |

# APPENDIX G

## STATISTICAL PROCEDURES FOR TESTING HOMOGENEITY OF TWO REGRESSIONS (F-TEST)

APPENDIX G

## STATISTICAL PROCEDURES FOR TESTING HOMOGENEITY OF TWO REGRESSIONS

This appendix is a summary of the statistical procedures to be used for testing homogeneity of two regressions. The logic is derived from an article entitled "Tests of Equality Between Sets of Coefficients in Two Linear Regressions," by Gregory Chow in Econometrica, July 1960.

**The model of normal linear regression has been widely applied to the measurement of functional relationships. When the linear regression is used to represent any functional relationship, the question often arises as to whether the relationship remains stable in two different experiments, or whether the same relationship holds for two periods of time. Statistically, these questions can be answered by testing whether two sets of observations can be regarded as belonging to the same regression model.**

**To state our problems more formally, let $y$ be the dependent variable, and $x_1, x_2, \ldots, x_p$ be the explanatory variables. Assume that there is a sample of n observations. These observations are governed by a model of normal linear regression. In matrix notations, the model is:**

$$\begin{bmatrix} y_1 \\ y_2 \\ \cdot \\ \cdot \\ \cdot \\ y_n \end{bmatrix} = \begin{bmatrix} x_{11}\, x_{12} \ldots x_{1p} \\ x_{21}\, x_{22} \ldots x_{2p} \\ \\ \cdot \quad \cdot \quad \cdot \\ \\ x_{n1}\, x_{n2} \ldots x_{np} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \cdot \\ \cdot \\ \cdot \\ \beta_p \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \cdot \\ \cdot \\ \cdot \\ \varepsilon_n \end{bmatrix}.$$

**Here the $x$'s are p fixed variates. The $\beta$'s are the regression coefficients - $\beta_1$ is the intercept if $x_1$ is set identically equal to one. The $\varepsilon$'s are independent and normally distributed, each with mean zero and standard deviation $\sigma$. Assuming n>p and nonsingularity of the X matrix, we can estimate the parameters $\beta_1, \beta_2, \ldots, \beta_p$ and $\sigma$. Our problems are the testing of whether m additional observations are from the same regression as the first sample of n observations, and the testing of whether subsets of coefficients in the two regressions are identical. The paper is devoted to a systematic and unified treatment of these tests.**

**To test the hypothesis that both samples belong to the same regression, the analysis of covariance can be used when m>p. The procedure of testing the homogeniety of the entire sets of coefficients in two regressions can be described as follows:**

Assume that the first and the second sample sizes are n and m, respectively, and n and m are larger than p.

The model of general linear hypothesis takes the form

(1)
$$\begin{aligned} y_1 &= X_1\beta_1 + 0\,\beta_2 + \varepsilon_1 \\ y_2 &= 0\,\beta_1 + X_2\beta_2 + \varepsilon_2 \end{aligned}$$

or
$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}.$$

Under the null hypothesis ( $H_o: \beta_1 = \beta_2 = \beta$ ), the model becomes

(2)
$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} \beta + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}.$$

The sum of squares of the residuals under $H_o$ will be shown to equal the sum of squares of residuals under the alternative hypothesis ( $H_a: \beta_1 \neq \beta_2$ ) plus the sum of squares of the deviations between the two sets of estimates of $y$ under these two hypotheses. The ratio between the latter two sums, adjusted for their numbers of degrees of freedom, will be shown to follow an F distribution if the null hypothesis is true.

If the null hypothesis is true, the least-squares (also maximum likelihood) estimator of $\beta$ , denoted by $b_o$ , is

(3)
$$b_o = \left[ (X_1' X_2') \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \right]^{-1} [X_1' X_2'] \begin{bmatrix} y_1 \\ y_2 \end{bmatrix}$$
$$= [X_1'X_1 + X_2'X_2]^{-1} [X_1' X_2'] \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \beta + [X_1'X_1 + X_2'X_2]^{-1} [X_1' X_2'] \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}.$$

The residuals from this regression are:

(4)
$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} - \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} b_o = \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} \beta + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix} - \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} \beta$$
$$- \begin{bmatrix} X_1 \\ X_2 \end{bmatrix} [X_1'X_1 + X_2'X_2]^{-1} [X_1' X_2'] \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}$$
$$= \left[ I - \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} (X_1'X_1 + X_2'X_2)^{-1} (X_1' X_2') \right] \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}.$$

The sum of squares of the residuals under $H_o$ can be written as

(5)
$$\left\| \begin{pmatrix} y_1 \\ y_2 \end{pmatrix} - \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} b_o \right\|^2 = \left[ \begin{pmatrix} y_1 \\ y_2 \end{pmatrix} - \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} b_o \right]' \left[ \begin{pmatrix} y_1 \\ y_2 \end{pmatrix} - \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} b_o \right]$$
$$= [\varepsilon_1' \varepsilon_2'] \left[ I - \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} (X_1'X_1 + X_2'X_2)^{-1} (X_1' X_2') \right] \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}.$$

Since these residuals are from a regression of n+m observations on p explanatory variables, the quadratic form (5) in the $\varepsilon$'s has rank n+m-p.

If the alternative hypothesis ( $H_a$: $\beta_1 \neq \beta_2$) is true, we are back to the model (1), and the least-squares estimators of $\beta_1$ and $\beta_2$ are

$$(6) \qquad \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} = \begin{bmatrix} X_1'X_1 & 0 \\ 0 & X_2'X_2 \end{bmatrix}^{-1} \begin{bmatrix} X_1' & 0 \\ 0 & X_2' \end{bmatrix} \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} (X_1'X_1)^{-1} X_1' y_1 \\ (X_2'X_2)^{-1} X_2' y_2 \end{bmatrix}.$$

The residuals under will be

$$(7) \qquad \begin{bmatrix} y_1 - X_1 b_1 \\ y_2 - X_2 b_2 \end{bmatrix} = \begin{bmatrix} [I - X_1 (X_1'X_1)^{-1} X_1'] e_1 \\ [I - X_2 (X_2'X_2)^{-1} X_2'] e_2 \end{bmatrix}.$$

Similarly, the sum of squares of these residuals will be

$$(8) \qquad \left\| \begin{matrix} y_1 - X_1 b_1 \\ y_2 - X_2 b_2 \end{matrix} \right\|^2 = \|y_1 - X_1 b_1\|^2 + \|y_2 - X_2 b_2\|^2$$

$$= \varepsilon_1'[I - X_1(X_1'X_1)^{-1} X_1']\varepsilon_1 + \varepsilon_2'[I - X_2(X_2'X_2)^{-1} X_2']\varepsilon_2 .$$

Since the last two quadratic forms have ranks n-p and m-p respectively, and since $\varepsilon_1$ and $\varepsilon_2$ are independent, the rank of the quadratic form (8) will be n+m-2p.

Now the sum of squares (5) under $H_o$ will be decomposed into the sum of squares (8) under $H_a$ plus the sum of squares of the differences

$$[X_1 b_1 - X_1 b_o] \text{ and } [X_2 b_2 - X_2 b_o].$$

First start from the identity

$$(9) \qquad \begin{bmatrix} y_1 - X_1 b_o \\ y_2 - X_2 b_o \end{bmatrix} = \begin{bmatrix} y_1 - X_1 b_1 \\ y_2 - X_2 b_2 \end{bmatrix} + \begin{bmatrix} X_1 b_1 - X_1 b_o \\ X_2 b_2 - X_2 b_o \end{bmatrix}.$$

Summing the squares of the elements on both sides of (9) gives

$$(10) \qquad \left\| \begin{matrix} y_1 - X_1 b_o \\ y_2 - X_2 b_o \end{matrix} \right\|^2 = \left\| \begin{matrix} y_1 - X_1 b_1 \\ y_2 - X_2 b_2 \end{matrix} \right\|^2 + \left\| \begin{matrix} X_1 b_1 - X_1 b_o \\ X_2 b_2 - X_2 b_o \end{matrix} \right\|^2$$

because the cross-product term on the right side of (10) can easily be seen to be zero. To economize space, (10) will also be written as

$$(11) \qquad Q_1 = Q_2 + Q_3 .$$

Under the null hypothesis $\beta_1 = \beta_2 = \beta$, $Q_3$ will thus be a quadratic form in the $\varepsilon$'s with a maximum rank of p. We also see that $Q_3$ will tend to be larger when the null hypothesis is not true.

It has already been observed that the rank of $Q_3$ is m+n-2p. Since the rank of $Q_1$ is smaller than or equal to the rank of $Q_2$ plus the rank of $Q_3$, the rank of $Q_2$ must be p. Under the null hypothesis $Q_2$ and $Q_3$ will be distributed independently as $\chi^2(m+n-2p)\sigma^2$ and $\chi^2(p)\sigma^2$. While the distribution of $Q_3$ is affected if $H_0$ does not hold, $Q_2$ will have the same distribution regardless. We thus can test $H_0$ by the F ratio

(12)
$$F(p, m+n-2p) = \frac{Q_2/p}{Q_3/(m+n-2p)}$$
$$= \frac{\|X_1 b_1 - X_1 b_0\|^2 + \|X_2 b_2 - X_2 b_0\|^2}{\| y_1 - X_1 b_1 \|^2 + \| y_2 - X_2 b_2 \|^2} \cdot \frac{(m+n-2p)}{p}.$$

(12) is the standard analysis-of-covariance test when m>p.

We will now proceed with the analysis of covariance (12). The method involved can be described very simply. Suppose that n observations are used to estimate a regression with p parameters (p-1 coefficients plus one intercept). Suppose also that there are m additional observations, and we are interested in deciding whether they are generated by the same regression model as the first n observations. To perform the analysis of covariance, we need the following sums of squares:

A, sum of squares of n+m deviations of the dependent variable from the regression estimated by n+m observations, with n+m-p degrees of freedom.
B, sum of squares of n deviations of the dependent variable from the regression estimated by the first n observations, with n-p degrees of freedom.
C, sum of squares of m deviations of the dependent variable from the regression estimated by the second m observations, with m-p degrees of freedom.

From (12), the ratio of (A-B-C)/p to (B+C)/(n+m-2p) will be distributed as F(p,n+m-2p) under the null hypothesis that both groups of observations belong to the same regression model.

# APPENDIX H

# NITROGEN MASS BALANCE OF CONTINUOUS FLOW SOIL COLUMNS

APPENDIX H

TOTAL NITROGEN MASS BALANCE

Influent Wastewater = Effluent Wastewater and (Soil Adsorption)
Soil Adsorption = Post-Treatment Soil - Original Soil

Column 1

63.75 = 85.03 + (753 - 774.67)

63.75 = 63.36
99%

Column 2

2402.53 = 1773.35 + (1021.5 - 774.67)

2402.53 = 2020.18
84%

Column 3

2011.29 = 1557.40 + (1361.5 - 774.67)

2011.29 = 2144.23
107%

Column 4

1991.88 = 1297.53 + (1640 - 774.67)

1991.88 = 2162.86
109%

Column 5

2067.22 = 1152.81 + (1605 - 774.67)

2067.22 = 1983.14
96%

Column 6

1975.19 = 1198.08 + (1285.5 - 774.67)

1975.19 = 1708.91
87%

Continuous Flow SFAAP Soil Column Nitrogen Balance

Column 1 Influent

| Day(s) | 10 | 38 | 46 | 69 | 90 | 116 | 176 | 204 | 232 | 245 | 260 | 271 | Total mg | Nitrogen mg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 2.70 | 0.77 | 2.20 | 2.00 | 2.70 | 5.60 | 2.70 | 2.70 | 1.20 | 1.40 | 1.10 | | | |
| NO | 0.00 | 0.00 | 96.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 96.00 | 0.538 | 51.65 |
| NOO | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.638 | 0.00 |
| CY | 0.00 | 0.00 | 5.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.38 | 0.670 | 3.60 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.710 | 0.00 |
| CYG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.667 | 0.00 |
| NO2-NO3 | 0.00 | 10.18 | 9.89 | 0.00 | 0.00 | 2.78 | 0.00 | 0.19 | 1.54 | 0.00 | 0.00 | 0.00 | 24.58 | 0.260 | 6.39 |
| NH3-N | 0.00 | 0.00 | 2.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.32 | 0.00 | 0.00 | 0.00 | 2.57 | 0.823 | 2.11 |
| | | | | | | | | | | | | | | | 63.75 |

Column 1 Effluent

| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | Total mg | Nitrogen mg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NO | 0.00 | 0.00 | 13.06 | 63.84 | 9.22 | 0.54 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 86.78 | 0.538 | 46.68 |
| NOO | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.638 | 0.00 |
| CY | 0.00 | 0.00 | 0.00 | 3.23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.36 | 0.00 | 0.00 | 0.00 | 0.00 | 4.32 | 0.00 | 0.00 | 10.91 | 0.670 | 7.31 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.710 | 0.00 |
| NO2-NO3 | 2.69 | 11.00 | 6.60 | 8.33 | 0.00 | 0.00 | 4.84 | 5.30 | 0.00 | 3.90 | 3.56 | 1.17 | 0.00 | 0.00 | 20.80 | 5.20 | 0.00 | 0.00 | 7.20 | 6.30 | 7.70 | 94.59 | 0.260 | 24.59 |
| NH3-N | 0.25 | 0.03 | 0.03 | 0.00 | 0.00 | 7.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 7.84 | 0.823 | 6.45 |
| | | | | | | | | | | | | | | | | | | | | | | | | 85.03 |

Influent

Continuous Flow SFAAP Soil Column Nitrogen Balance

| Column 2 Influent | | | | | | | | | | | | | Total | | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day(s) | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 | mg | | mg |
| Volume | 0.96 | 2.70 | 0.77 | 2.20 | 2.00 | 2.70 | 5.60 | 2.70 | 2.70 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 127.68 | 267.30 | 104.45 | 374.00 | 13.40 | 367.20 | 728.00 | 550.80 | 348.30 | 159.60 | 156.20 | 126.50 | 3325.43 | 0.538 | 1789.08 |
| NOQ | 0.08 | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.45 | 0.636 | 0.29 |
| CY | 17.28 | 27.00 | 5.76 | 18.04 | 15.20 | 23.49 | 36.40 | 361.80 | 27.00 | 12.96 | 15.40 | 11.33 | 571.66 | 0.670 | 383.01 |
| G | 11.23 | 0.00 | 8.06 | 17.38 | 0.00 | 20.79 | 50.96 | 26.19 | 0.00 | 0.00 | 0.00 | 0.00 | 134.62 | 0.710 | 95.56 |
| CYG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.667 | 0.00 |
| NO2-NO3 | 1.34 | 48.87 | 8.45 | 29.04 | 28.80 | 26.73 | 84.00 | 43.20 | 29.70 | 13.20 | 14.42 | 11.33 | 339.08 | 0.260 | 88.16 |
| NH3-N | 2.11 | 0.46 | 3.58 | 6.38 | 5.74 | 6.21 | 12.88 | 7.02 | 3.51 | 2.81 | 2.91 | 2.78 | 56.39 | 0.823 | 46.41 |
| | | | | | | | | | | | | | | | 2402.53 |

Influent

| Column 2 Effluent | | | | | | | | | | | | | | | | | | | | | Total | Nitrogen | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | mg | mg | |
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 10.75 | 15.10 | 53.76 | 90.05 | 102.91 | 115.97 | 96.08 | 159.74 | 100.13 | 95.02 | 81.98 | 165.36 | 174.20 | 504.00 | 178.10 | 183.30 | 164.40 | 0.00 | 153.60 | 159.60 | 204.60 | 2608.65 | 0.538 | 1511.06 |
| NOQ | 0.02 | 0.00 | 0.17 | 0.20 | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.10 | 0.00 | 0.00 | 0.00 | 0.39 | 0.59 | 0.36 | 0.00 | 0.00 | 0.00 | 12.76 | 14.86 | 0.636 | 9.45 |
| CY | 3.17 | 0.00 | 9.98 | 5.24 | 5.38 | 5.76 | 4.95 | 5.22 | 5.64 | 5.38 | 5.71 | 11.83 | 10.01 | 36.12 | 9.10 | 12.74 | 12.00 | 0.00 | 15.84 | 15.68 | 12.76 | 192.52 | 0.670 | 128.99 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 9.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 9.45 | 0.710 | 6.71 |
| NO2-NO3 | 2.30 | 11.00 | 19.74 | 0.94 | 1.23 | 6.60 | 4.67 | 5.53 | 16.53 | 13.24 | 7.39 | 8.58 | 11.44 | 80.64 | 18.20 | 39.00 | 10.60 | 162.40 | 8.40 | 9.66 | 7.48 | 445.77 | 0.260 | 115.90 |
| NH3-N | 0.21 | 0.00 | 0.04 | 0.00 | 0.12 | 0.10 | 0.13 | 0.07 | 0.09 | 0.00 | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.29 | 0.31 | 0.00 | 1.52 | 0.823 | 1.25 |
| | | | | | | | | | | | | | | | | | | | | | | | | 1773.35 |

Continuous Flow SFAAP Soil Column Nitrogen Balance

Column 3 Influent

| Day(s) | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 | Total mg | | Nitrogen mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 2.70 | 0.77 | 2.20 | 2.00 | 2.70 | 5.60 | 2.70 | 2.70 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 125.76 | 256.50 | 96.77 | 334.40 | 2.80 | 341.28 | 672.00 | 356.40 | 356.40 | 157.20 | 170.80 | 137.50 | 3007.81 | 0.538 | 1618.20 |
| NOQ | 0.00 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 0.636 | 0.14 |
| CY | 29.76 | 27.00 | 15.36 | 0.00 | 14.60 | 0.00 | 0.00 | 0.00 | 0.00 | 3.00 | 2.80 | 4.40 | 96.92 | 0.670 | 64.94 |
| G | 9.70 | 0.00 | 7.45 | 31.46 | 0.00 | 27.54 | 39.76 | 20.52 | 17.01 | 6.96 | 8.54 | 0.00 | 168.94 | 0.710 | 119.94 |
| CYG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.667 | 0.00 |
| | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.260 | 0.00 |
| NO2-NO3 | 0.29 | 4.05 | 1.46 | 48.40 | 51.20 | 10.80 | 26.88 | 16.20 | 40.50 | 8.40 | 9.38 | 6.16 | 223.72 | 0.823 | 184.12 |
| NH3-N | 0.00 | 4.62 | 2.11 | 2.42 | 1.00 | 1.62 | 2.80 | 0.00 | 12.04 | 0.00 | 0.00 | 0.00 | 26.61 | 0.900 | 23.95 |
| | | | | | | | | | | | | | | | 2011.29 |

Influent

Column 3 Effluent

| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | Total mg | | Nitrogen mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 0.38 | 100.00 | 86.78 | 70.56 | 65.25 | 106.75 | 73.79 | 86.02 | 0.60 | 84.54 | 75.26 | 145.60 | 141.57 | 462.00 | 397.80 | 157.30 | 158.40 | 0.00 | 134.40 | 148.40 | 124.30 | 2639.71 | 0.538 | 1420.16 |
| NOQ | 0.00 | 0.20 | 0.12 | 0.54 | 0.84 | 0.00 | 0.00 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.01 | 0.636 | 1.28 |
| CY | 2.30 | 5.50 | 4.38 | 4.30 | 5.38 | 0.00 | 8.12 | 0.00 | 13.51 | 10.95 | 4.84 | 0.00 | 4.29 | 6.72 | 0.00 | 0.00 | 12.00 | 0.00 | 9.36 | 0.00 | 0.00 | 91.65 | 0.670 | 61.40 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.28 | 0.00 | 7.15 | 17.64 | 0.00 | 4.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 31.62 | 0.710 | 22.45 |
| NO2-NO3 | 2.30 | 1.50 | 1.54 | 0.54 | 0.84 | 2.23 | 8.87 | 0.15 | 16.80 | 10.82 | 4.97 | 6.11 | 7.02 | 20.16 | 7.80 | 7.80 | 9.60 | 5.60 | 8.40 | 7.56 | 5.39 | 136.01 | 0.260 | 35.36 |
| NH3-N | 1.34 | 1.93 | 1.96 | 0.68 | 0.52 | 0.28 | 0.23 | 0.00 | 0.34 | 0.00 | 0.20 | 0.52 | 1.69 | 6.30 | 1.56 | 1.56 | 0.00 | 0.00 | 0.00 | 0.00 | 1.23 | 20.35 | 0.823 | 16.75 |
| | | | | | | | | | | | | | | | | | | | | | | | | 1557.41 |

Continuous Flow SFAAP Soil Column Nitrogen Balance

Column 4 Influent

| | | | | | | | | | | | | | Total | | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 2.70 | 0.77 | 2.20 | 2.00 | 2.70 | 5.60 | 2.70 | 2.70 | 1.20 | 1.40 | 1.10 | | | |
| Day(s) | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 | mg | | mg |
| NO | 113.28 | 194.40 | 99.84 | 345.40 | 13.40 | 351.00 | 672.00 | 378.00 | 364.50 | 159.60 | 170.80 | 125.40 | 2987.62 | 0.538 | 1607.34 |
| NOO | 0.00 | 0.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.54 | 0.00 | 0.00 | 1.43 | 2.43 | 0.638 | 1.54 |
| CY | 11.52 | 19.71 | 5.07 | 7.48 | 13.60 | 21.60 | 36.96 | 18.63 | 27.00 | 12.48 | 14.42 | 11.33 | 199.80 | 0.670 | 133.87 |
| G | 10.18 | 0.00 | 8.68 | 25.96 | 17.20 | 22.41 | 44.80 | 23.22 | 0.00 | 0.00 | 0.00 | 0.00 | 152.44 | 0.710 | 108.24 |
| CY6 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.59 | 3.48 | 0.00 | 0.00 | 8.07 | 0.667 | 5.38 |
| NO2-NO3 | 5.57 | 45.09 | 12.83 | 25.30 | 51.60 | 26.19 | 82.32 | 40.50 | 27.00 | 10.80 | 11.34 | 8.47 | 347.00 | 0.260 | 90.22 |
| NH3-N | 1.25 | 0.38 | 2.65 | 4.84 | 1.00 | 6.21 | 17.92 | 7.29 | 6.08 | 2.21 | 3.01 | 2.20 | 55.03 | 0.823 | 45.29 |
| | | | | | | | | | | | | | | | 1991.88 |

Influent

Column 4 Effluent

| | | | | | | | | | | | | | | | | | | | | | Total | | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | mg | | mg |
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NO | 0.67 | 58.00 | 94.46 | 84.00 | 96.77 | 107.52 | 67.39 | 33.79 | 7.32 | 45.56 | 43.55 | 53.30 | 12.87 | 546.00 | 73.58 | 158.60 | 51.60 | 0.00 | 175.20 | 118.02 | 81.40 | 1909.61 | 0.538 | 1027.37 |
| NOO | 0.00 | 0.10 | 0.29 | 0.40 | 0.61 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 3.51 | 0.26 | 4.08 | 0.00 | 0.00 | 0.98 | 0.66 | 10.95 | 0.638 | 6.97 |
| CY | 0.00 | 2.90 | 4.84 | 4.70 | 0.00 | 5.30 | 3.57 | 0.00 | 4.62 | 5.31 | 5.31 | 10.40 | 0.00 | 29.82 | 11.05 | 8.45 | 7.80 | 0.00 | 21.48 | 13.30 | 9.62 | 147.67 | 0.670 | 99.07 |
| G | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 34.44 | 0.00 | 17.55 | 0.00 | 0.00 | 6.84 | 0.00 | 0.00 | 59.13 | 0.710 | 41.98 |
| NO2-NO3 | 39.36 | 9.00 | 9.60 | 0.54 | 1.38 | 5.61 | 4.38 | 2.76 | 5.91 | 4.44 | 3.90 | 1.56 | 5.46 | 51.66 | 5.20 | 11.70 | 4.80 | 17.36 | 8.40 | 6.30 | 5.50 | 204.62 | 0.260 | 53.25 |
| NH3-N | 0.59 | 0.09 | 0.30 | 0.03 | 0.07 | 0.74 | 0.16 | 0.00 | 0.20 | 1.34 | 2.62 | 0.91 | 3.25 | 15.96 | 18.33 | 7.15 | 17.40 | 0.00 | 2.05 | 4.33 | 8.20 | 83.71 | 0.823 | 68.89 |
| | | | | | | | | | | | | | | | | | | | | | | | | 1297.53 |

Continuous Flow SFAAP Soil Column Nitrogen Balance

Column 5 Influent

| Day(s) | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 | Total ug | | Nitrogen ug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NO | 121.92 | 313.20 | 87.55 | 334.40 | 2.80 | 499.50 | 728.00 | 361.80 | 351.00 | 152.40 | 154.00 | 133.10 | 3239.67 | 0.538 | 1742.94 |
| NOO | 0.00 | 0.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.46 | 0.636 | 0.29 |
| CY | 0.15 | 35.10 | 13.82 | 9.68 | 20.60 | 21.60 | 0.00 | 0.00 | 0.00 | 2.40 | 0.00 | 18.26 | 121.62 | 0.670 | 81.48 |
| G | 8.64 | 0.00 | 7.14 | 54.78 | 0.00 | 39.15 | 47.04 | 24.84 | 16.74 | 8.88 | 7.98 | 6.80 | 223.99 | 0.710 | 159.03 |
| NO2-NO3 | 1.82 | 4.05 | 1.54 | 18.92 | 24.60 | 13.77 | 31.92 | 16.20 | 40.50 | 7.20 | 7.98 | 6.60 | 175.10 | 0.260 | 45.53 |
| NH3-N | 0.15 | 4.24 | 3.48 | 2.20 | 2.40 | 1.08 | 7.84 | 3.24 | 15.69 | 0.00 | 0.00 | 1.84 | 42.16 | 0.900 | 37.94 |
| | | | | | | | | | | | | | | | 2067.22 |

Influent

Column 5 Effluent

| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | Total ug | | Nitrogen ug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NO | 12.19 | 119.00 | 96.00 | 67.87 | 72.96 | 113.66 | 55.64 | 65.13 | 52.15 | 45.43 | 39.65 | 67.60 | 43.94 | 294.00 | 262.80 | 106.60 | 93.60 | 0.00 | 79.68 | 177.80 | 81.40 | 1887.10 | 0.538 | 1015.26 |
| NOO | 0.00 | 0.35 | 0.59 | 0.74 | 0.61 | 0.00 | 0.00 | 0.00 | 0.00 | 1.95 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 4.30 | 0.636 | 2.74 |
| CY | 3.55 | 5.50 | 5.38 | 3.76 | 4.53 | 1.38 | 0.85 | 0.00 | 6.99 | 2.08 | 5.38 | 0.00 | 0.00 | 0.00 | 12.48 | 12.22 | 0.00 | 0.00 | 0.00 | 0.00 | 6.71 | 70.82 | 0.670 | 47.45 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.63 | 0.710 | 4.71 |
| NO2-NO3 | 2.69 | 1.60 | 1.54 | 0.54 | 0.77 | 5.30 | 4.32 | 6.60 | 6.72 | 6.05 | 4.50 | 5.75 | 6.50 | 22.26 | 7.80 | 6.50 | 7.20 | 180.60 | 6.00 | 6.44 | 4.73 | 294.40 | 0.260 | 76.54 |
| NH3-N | 0.80 | 1.68 | 2.10 | 1.44 | 0.48 | 0.77 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 7.44 | 0.823 | 6.12 |
| | | | | | | | | | | | | | | | | | | | | | | | | 1150.81 |

Continuous Flow SFAAP Soil Column Nitrogen Balance

| Column 6 Influent | | | | | | | | | | | | | Total | | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day(s) | 10 | 38 | 46 | 69 | 90 | 118 | 176 | 204 | 232 | 245 | 260 | 271 | mg | | mg |
| Volume | 0.96 | 2.70 | 0.77 | 2.20 | 2.00 | 2.70 | 5.60 | 2.70 | 2.70 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 121.92 | 288.90 | 92.93 | 305.80 | 12.40 | 345.60 | 672.00 | 388.80 | 345.60 | 157.20 | 151.20 | 118.80 | 3001.15 | 0.538 | 1614.62 |
| NOQ | 0.00 | 1.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.57 | 0.636 | 1.00 |
| CY | 15.36 | 29.70 | 5.76 | 15.84 | 14.00 | 0.00 | 34.16 | 18.36 | 26.46 | 12.00 | 15.54 | 12.32 | 199.50 | 0.670 | 133.67 |
| G | 10.08 | 0.00 | 4.22 | 13.42 | 13.80 | 23.22 | 49.84 | 22.95 | 0.00 | 0.00 | 0.00 | 0.00 | 137.53 | 0.710 | 97.65 |
| CYG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.667 | 0.00 |
| NO2-NO3 | 2.21 | 3.24 | 2.07 | 22.66 | 16.60 | 23.22 | 73.36 | 37.80 | 10.80 | 9.60 | 10.36 | 6.60 | 218.52 | 0.260 | 56.82 |
| NH3-N | 4.32 | 1.11 | 3.65 | 7.85 | 12.16 | 9.18 | 13.27 | 10.53 | 9.37 | 3.23 | 6.48 | 5.67 | 86.82 | 0.823 | 71.45 |
| | | | | | | | | | | | | | | | 1975.19 |

Influent

| Column 6 Effluent | | | | | | | | | | | | | | | | | | | | | Total | | Nitrogen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day(s) | 10.00 | 38.00 | 46.00 | 53.00 | 61.00 | 69.00 | 75.00 | 83.00 | 90.00 | 97.00 | 104.00 | 118.00 | 132.00 | 176.00 | 190.00 | 204.00 | 217.00 | 232.00 | 245.00 | 260.00 | 271.00 | mg | | mg |
| Volume | 0.96 | 1.00 | 0.77 | 0.67 | 0.77 | 0.77 | 0.58 | 0.77 | 0.67 | 0.67 | 0.67 | 1.30 | 1.30 | 4.20 | 1.30 | 1.30 | 1.20 | 1.40 | 1.20 | 1.40 | 1.10 | | | |
| NQ | 10.37 | 68.00 | 52.99 | 22.85 | 33.79 | 108.29 | 10.14 | 14.82 | 25.20 | 16.67 | 2.15 | 70.20 | 67.99 | 714.00 | 55.64 | 126.10 | 93.60 | 0.00 | 46.20 | 158.20 | 64.72 | 1761.91 | 0.538 | 947.91 |
| NOQ | 0.65 | 0.00 | 1.31 | 1.21 | 4.38 | 0.00 | 3.59 | 0.00 | 0.00 | 2.62 | 0.00 | 0.00 | 0.00 | 0.00 | 5.07 | 0.00 | 1.80 | 0.00 | 0.00 | 0.00 | 0.00 | 20.03 | 0.636 | 12.74 |
| CY | 3.55 | 5.60 | 4.38 | 0.00 | 1.69 | 4.53 | 0.69 | 1.54 | 0.00 | 2.89 | 1.55 | 9.88 | 6.11 | 38.22 | 6.50 | 8.97 | 15.00 | 0.00 | 14.40 | 15.68 | 0.00 | 141.17 | 0.670 | 94.59 |
| G | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.710 | 0.00 |
| NO2-NO3 | 0.86 | 1.10 | 0.92 | 0.00 | 0.46 | 4.99 | 0.69 | 0.54 | 3.90 | 7.80 | 1.88 | 11.31 | 19.37 | 66.36 | 3.90 | 11.70 | 10.80 | 65.24 | 13.20 | 5.88 | 2.64 | 233.54 | 0.260 | 60.72 |
| NH3-N | 1.06 | 0.06 | 1.18 | 0.05 | 6.30 | 3.18 | 5.88 | 9.68 | 6.79 | 3.56 | 4.70 | 0.65 | 0.00 | 14.70 | 6.11 | 10.66 | 12.36 | 0.00 | 11.74 | 1.15 | 0.00 | 99.79 | 0.823 | 82.13 |
| | | | | | | | | | | | | | | | | | | | | | | | | 1198.08 |

# APPENDIX I

# TEMPERATURE RECORD OF SOIL COLUMNS

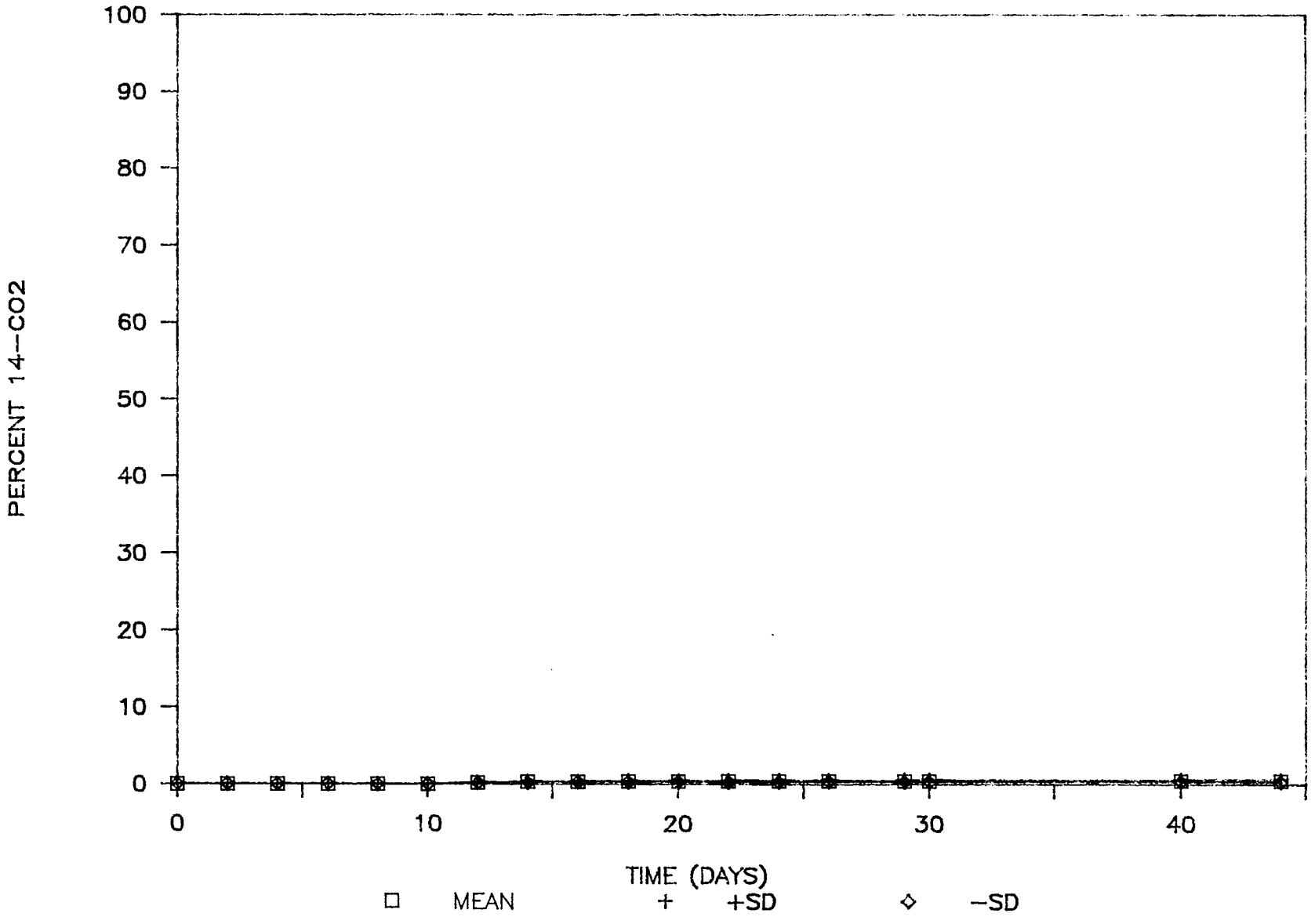
APPENDIX I

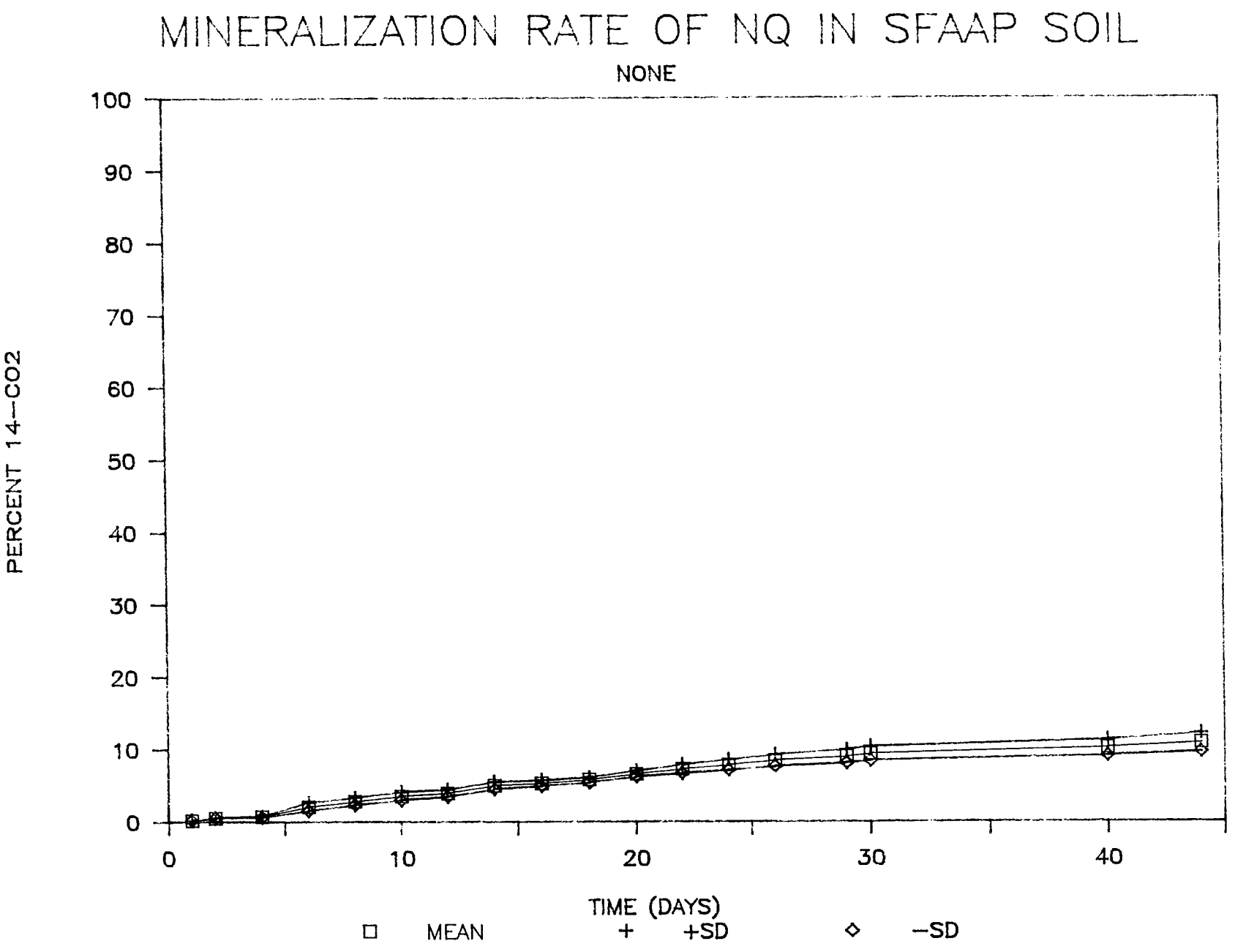
TEMPERATURE RECORDS OF CONTINUOUS FLOW
SOIL COLUMNS - SFAAP STUDY

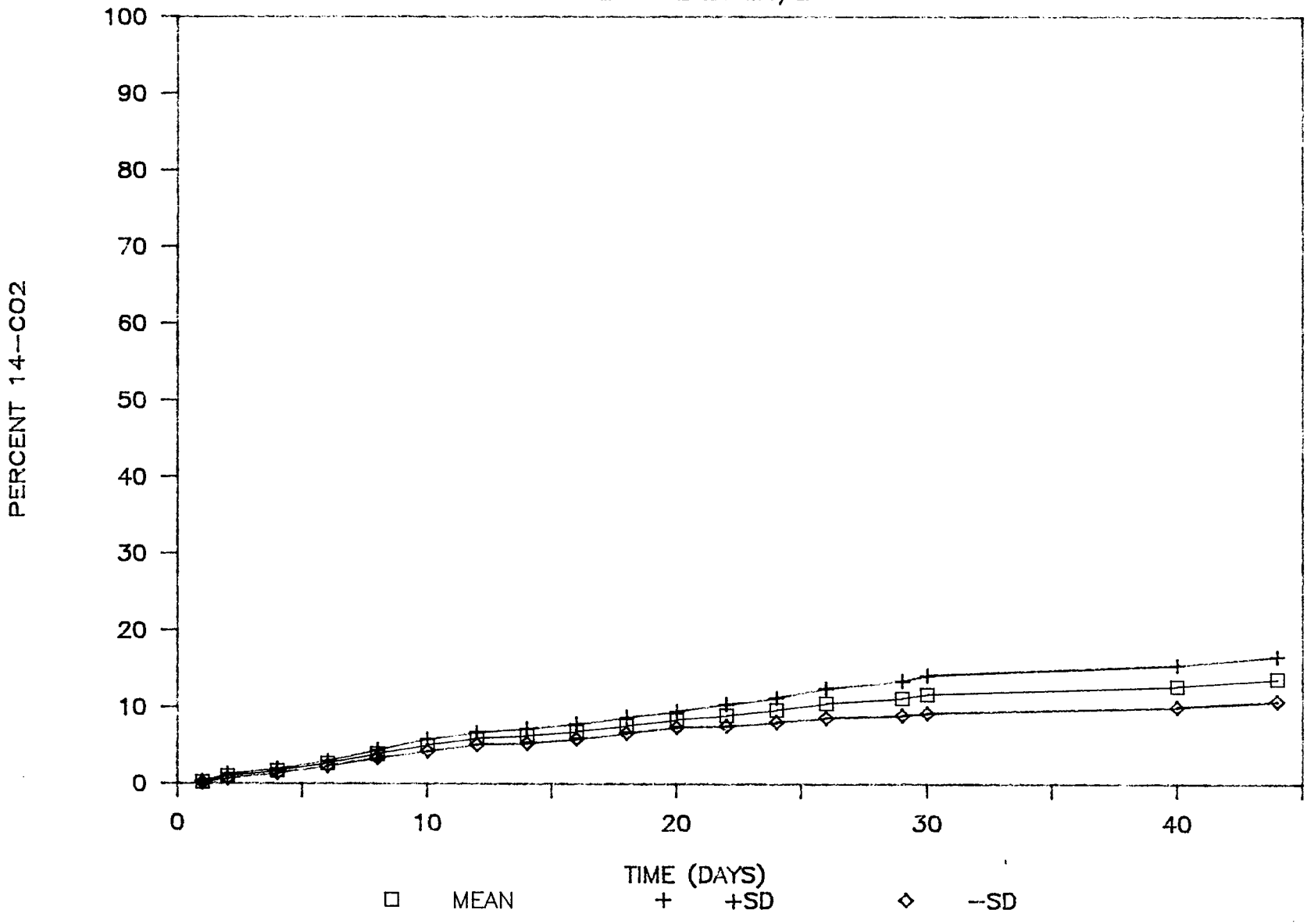
| Date | H | L | Date | H | L | |
|---|---|---|---|---|---|---|
| 12/20/85 | 20 | 18 | 06/11/86 | 25 | 23 | |
| 01/08/96 | 23 | 18 | 06/13/86 | 25 | 22 | Mean Temp - 23°C |
| 01/10/86 | 24 | 20 | 06/16/86 | 25 | 23 | |
| 01/15/86 | 25 | 18 | 06/18/86 | 25 | 23 | |
| 01/28/86 | 25 | 18 | 06/20/86 | 25 | 23 | |
| 02/10/86 | 25 | 17 | 06/23/86 | 25 | 23 | |
| 02/24/86 | 24 | 20 | 06/25/86 | 25 | 23 | |
| 03/01/86 | 24 | 20 | 06/27/86 | 25 | 22 | |
| 03/04/86 | 24 | 22 | 06/30/86 | 25 | 22 | |
| 03/06/86 | 24 | 20 | 07/02/86 | 25 | 23 | |
| 03/13/86 | 24 | 22 | 07/04/86 | 25 | 23 | |
| 03/17/86 | 24 | 18 | 07/07/86 | 25 | 23 | |
| 03/20/86 | 24 | 22 | 07/09/86 | 25 | 23 | |
| 04/03/86 | 24 | 22 | 07/11/86 | 26 | 23 | |
| 04/17/86 | 24 | 22 | 07/14/86 | 25 | 22 | |
| 05/01/86 | 25 | 23 | 07/16/86 | 26 | 24 | |
| 05/15/86 | 25 | 23 | 07/18/86 | 26 | 23 | |
| 05/19/86 | 26 | 23 | 07/21/86 | 26 | 23 | |
| 05/21/86 | 26 | 23 | 07/23/86 | 25 | 23 | |
| 05/23/86 | 25 | 23 | 07/25/86 | 26 | 24 | |
| 05/26/86 | 24 | 23 | 07/28/86 | 26 | 24 | |
| 05/28/86 | 24 | 23 | 07/30/86 | 26 | 23 | |
| 06/02/86 | 24 | 23 | 08/01/86 | 26 | 24 | |
| 06/04/86 | 24 | 22 | 08/04/86 | 26 | 23 | |
| 06/06/86 | 25 | 23 | 08/06/86 | 26 | 24 | |
| 06/09/86 | 25 | 23 | | | | |

APPENDIX J

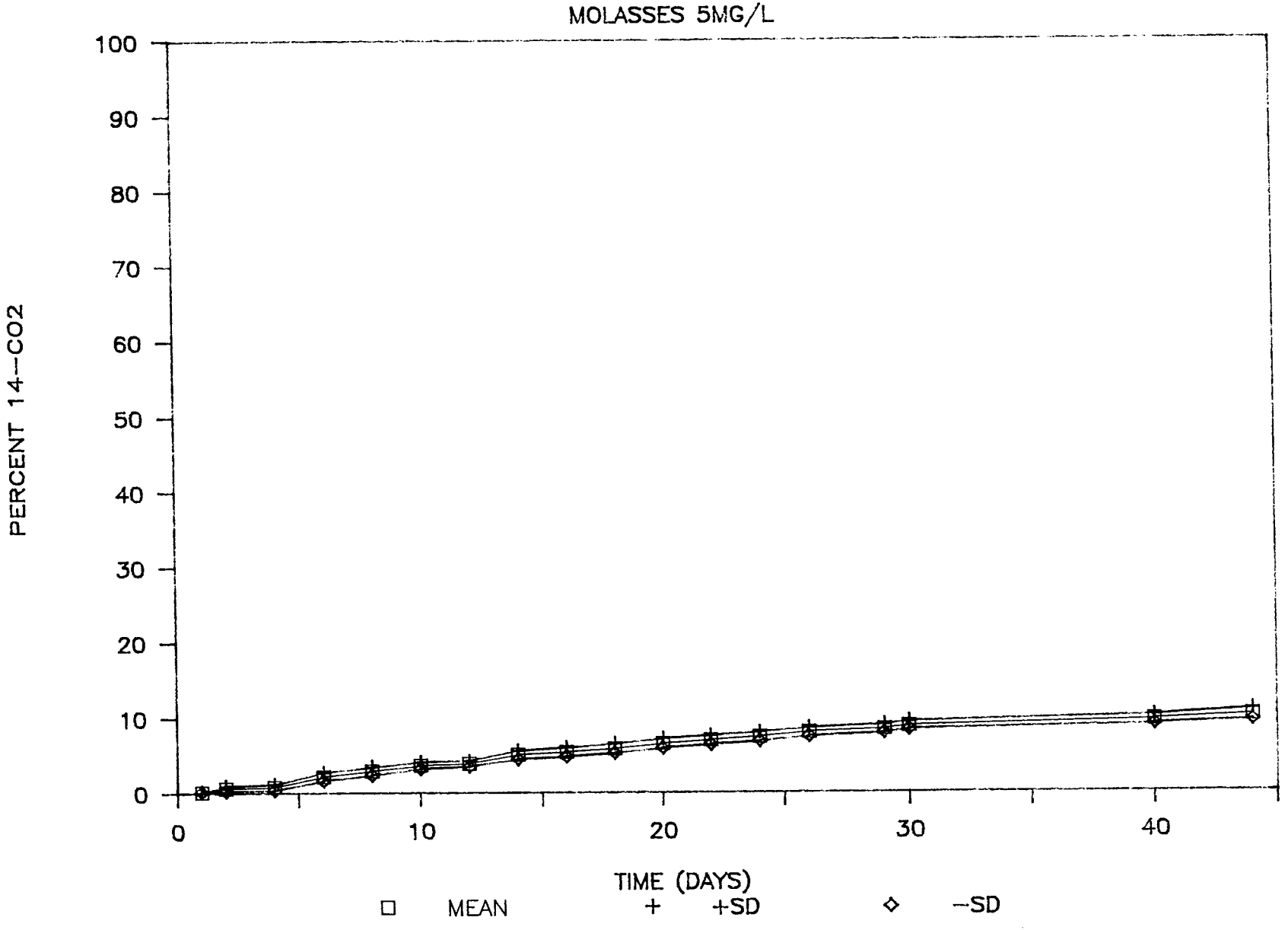
GRAPHS OF NQ AND GN MINERALIZATION IN PRETREATMENT SOIL VARIED CARBON SUPPLEMENT

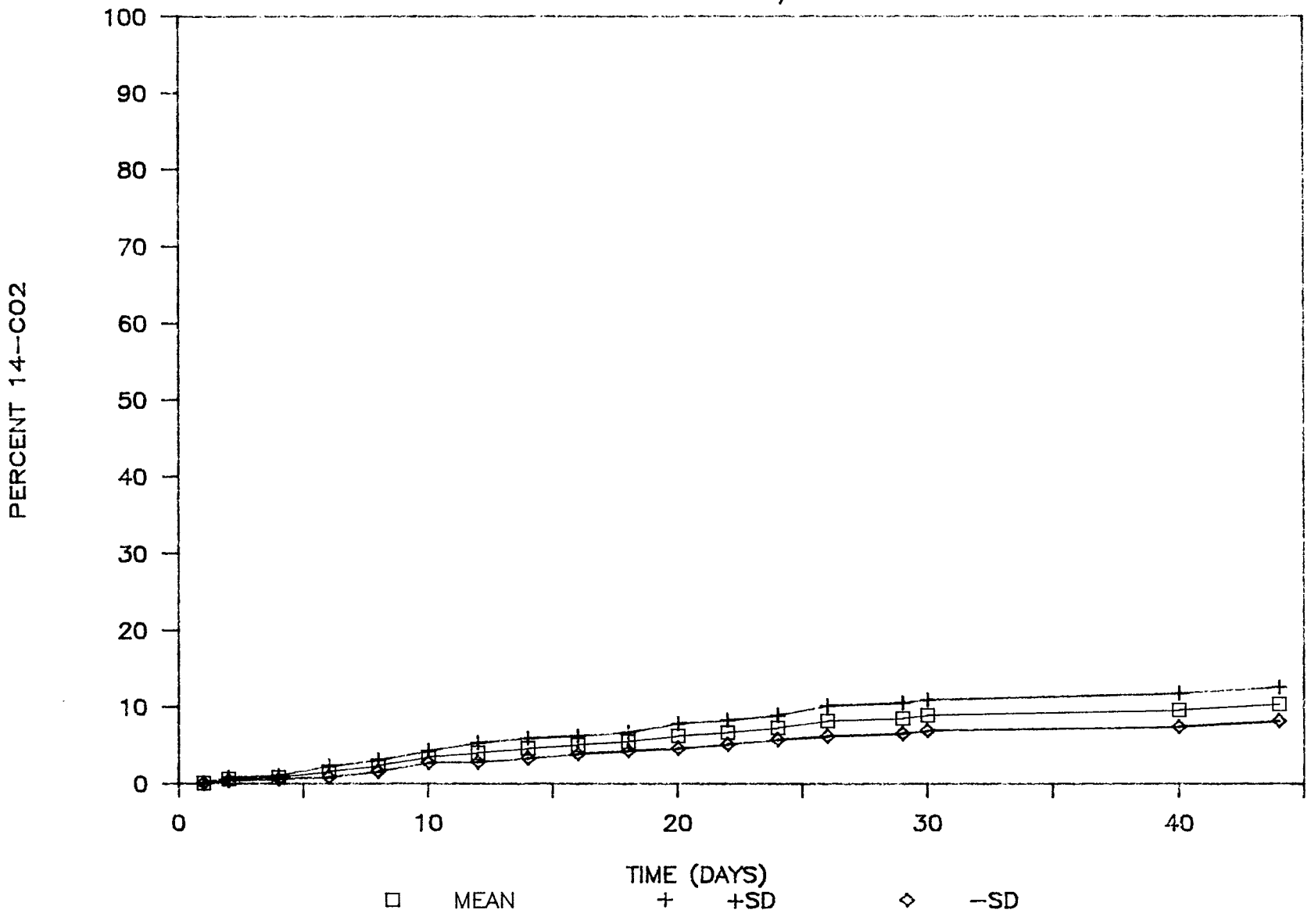

MINERALIZATION RATE OF NQ IN SFAAP SOIL
STERILE AUTOCLAVE
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD


MINERALIZATION RATE OF NQ IN SFAAP SOIL
NONE
PERCENT 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
—SD


MINERALIZATION RATE OF NQ IN SFAAP SOIL
GLUCOSE 20 MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
--SD

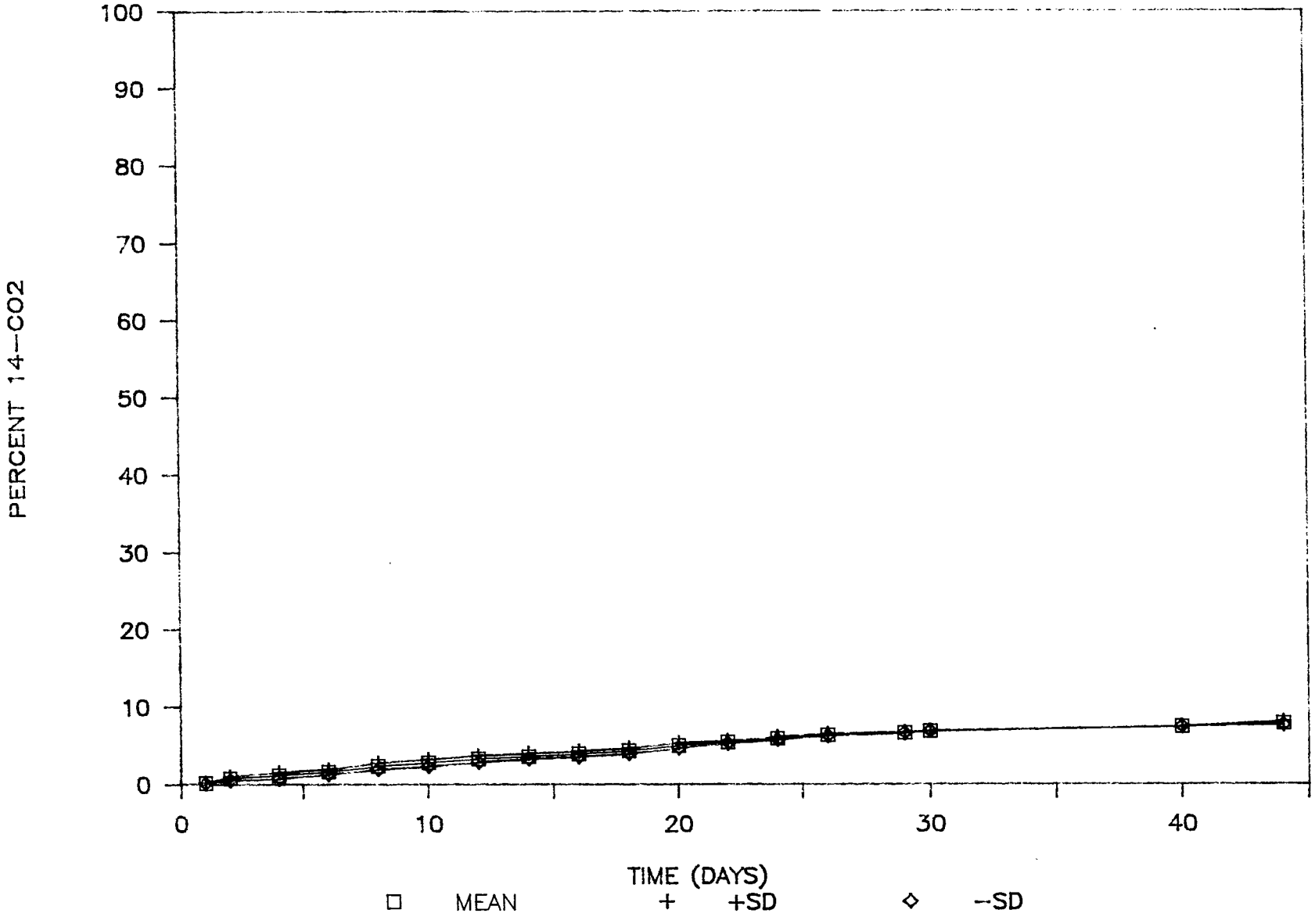
# MINERALIZATION RATE OF NQ IN SFAAP SOIL

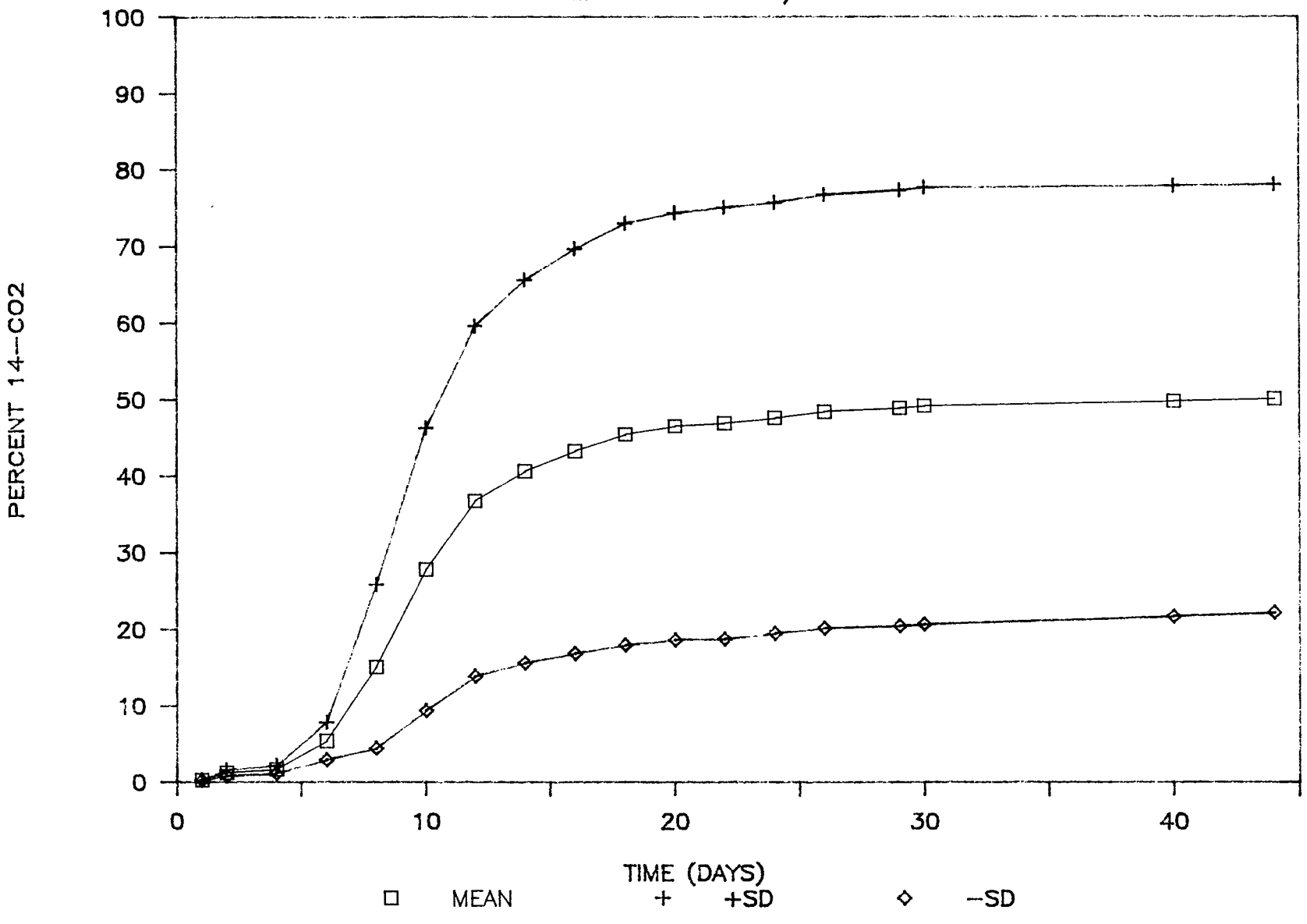



MINERALIZATION RATE OF NQ IN SFAAP SOIL
MOLASSES 20MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

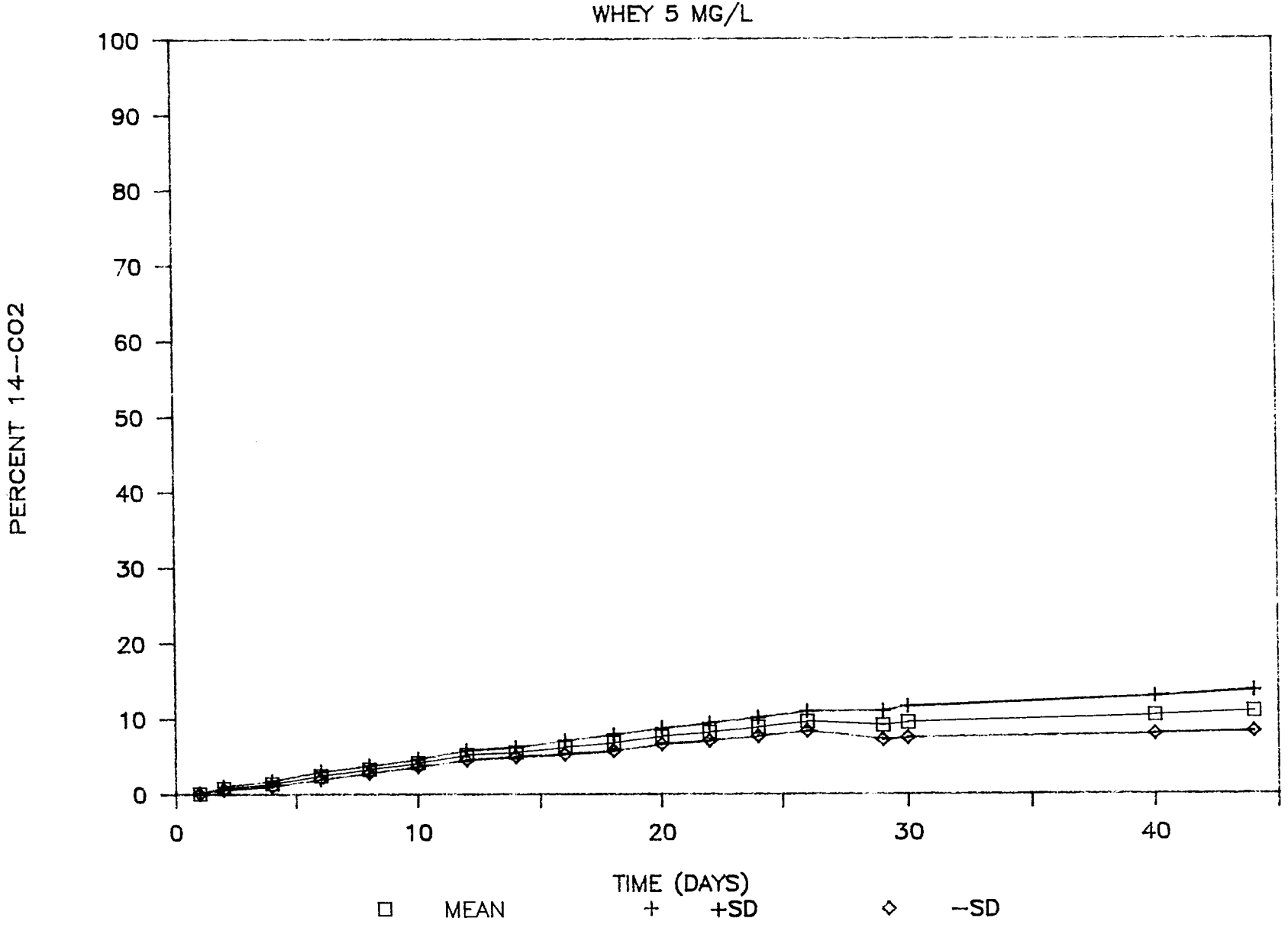
# MINERALIZATION RATE OF NQ IN SFAAP SOIL

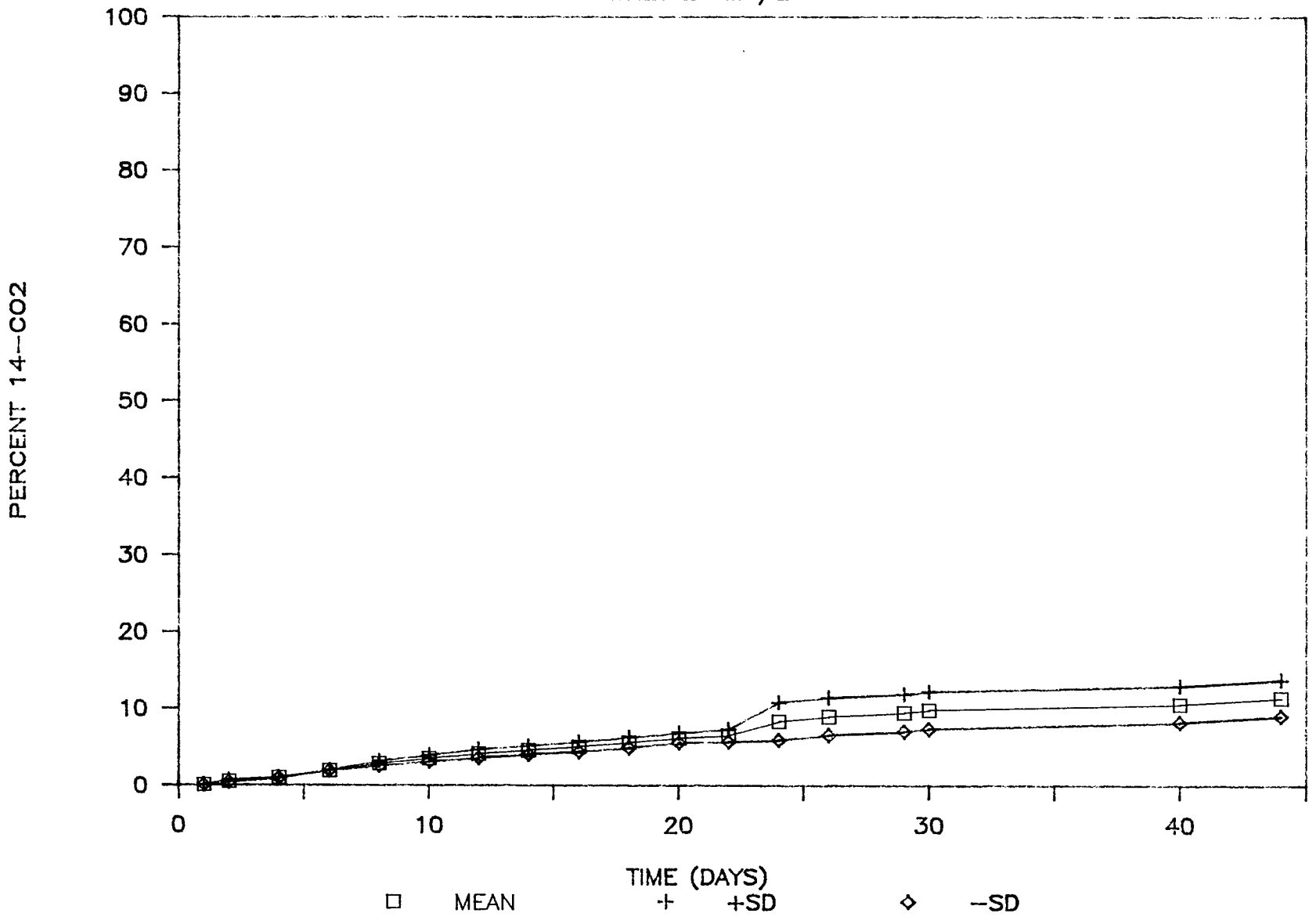
MOLASSES 100MG/L

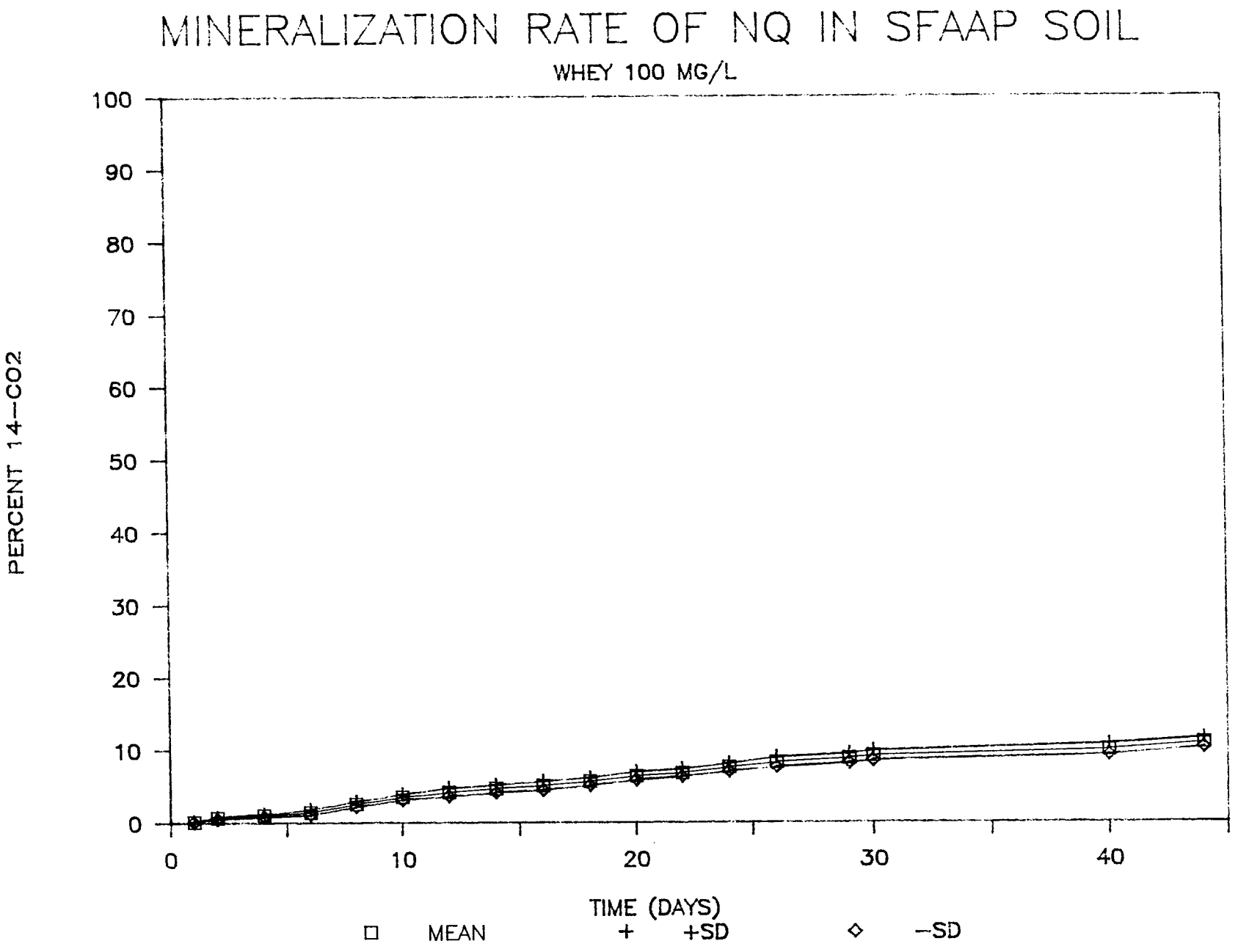


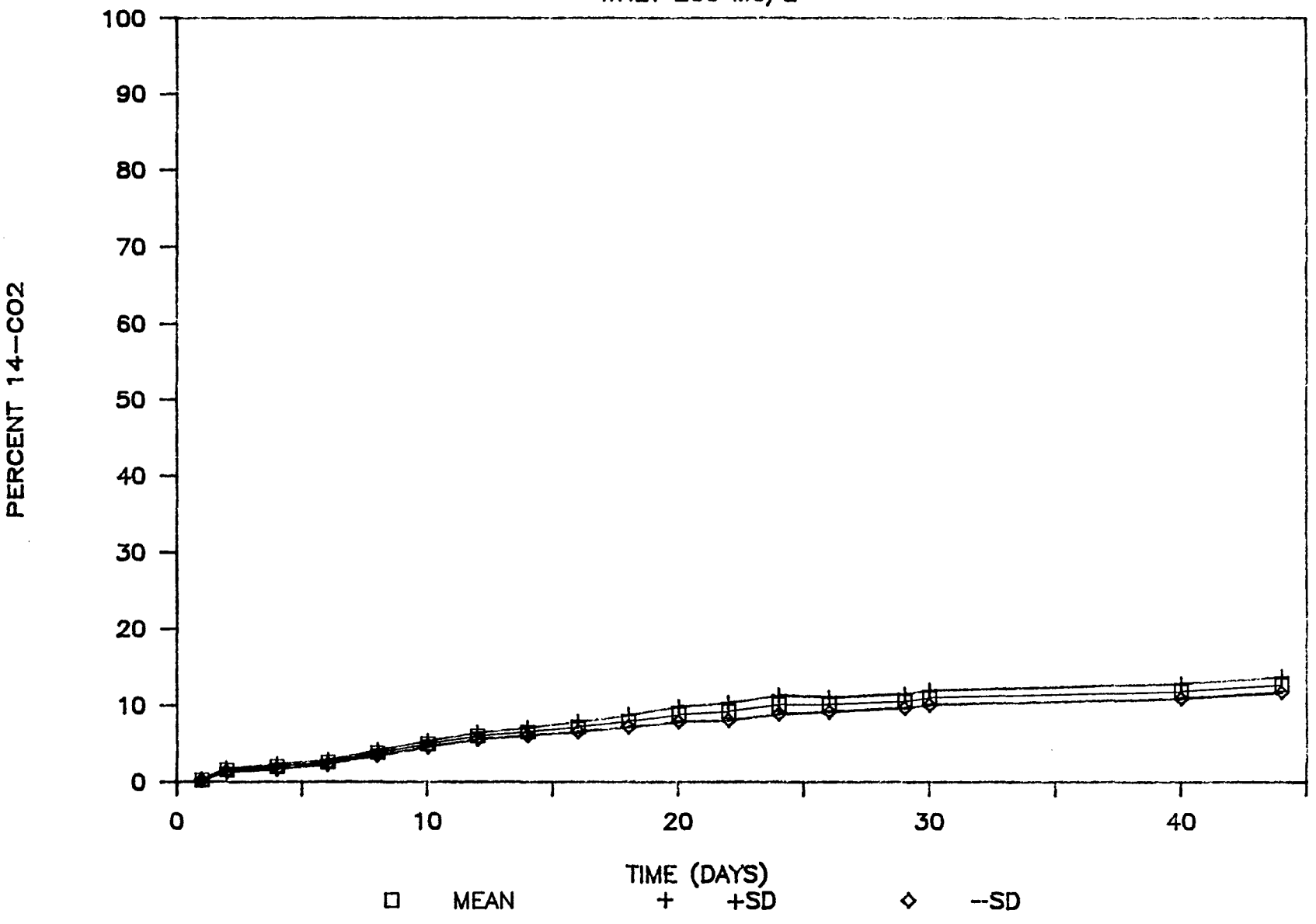

MINERALIZATION RATE OF NQ IN SFAAP SOIL
MOLASSES 200MG/L
PERCENT 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
—SD

# MINERALIZATION RATE OF NQ IN SFAAP SOIL



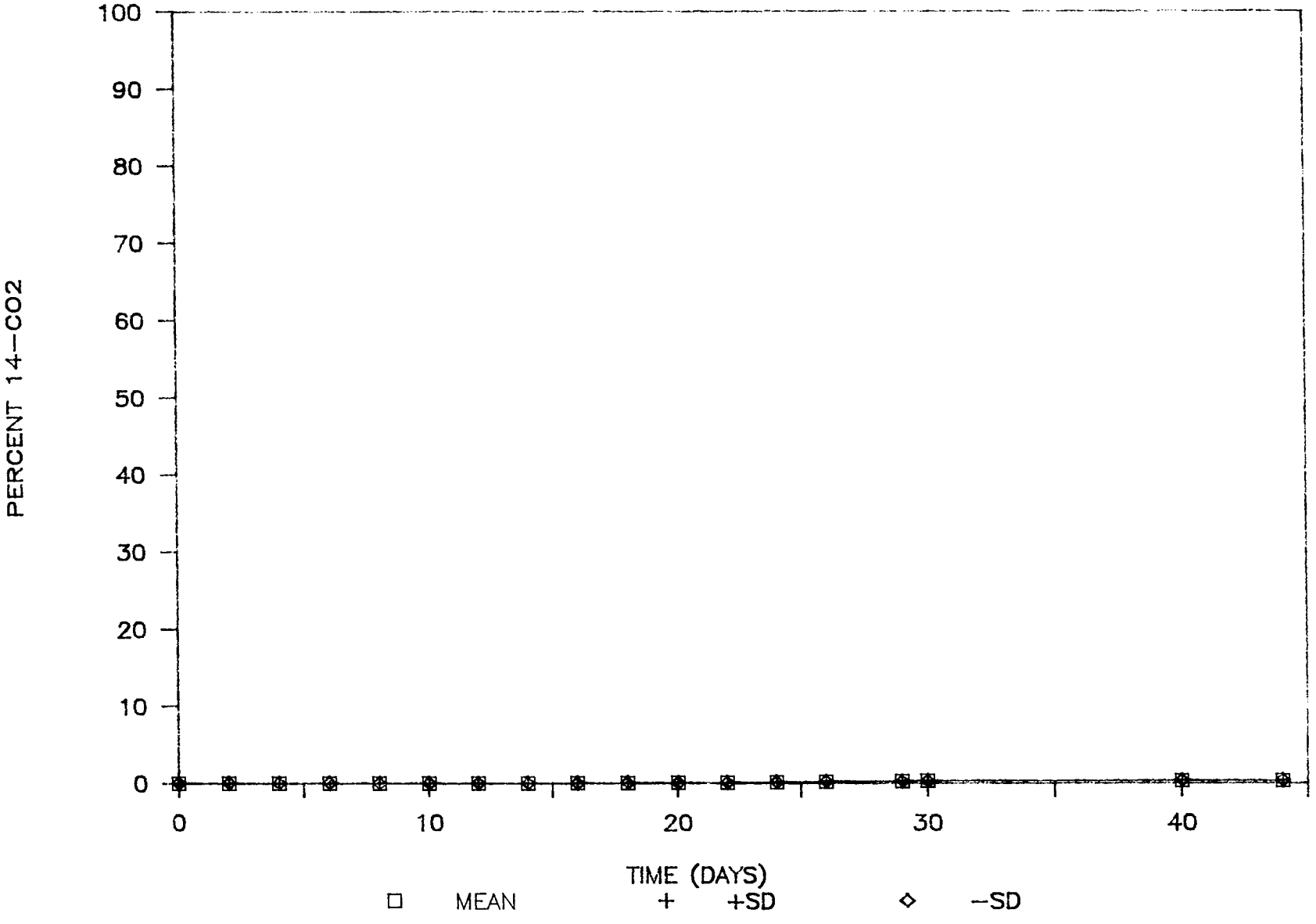

MINERALIZATION RATE OF NQ IN SFAAP SOIL
WHEY 20 MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

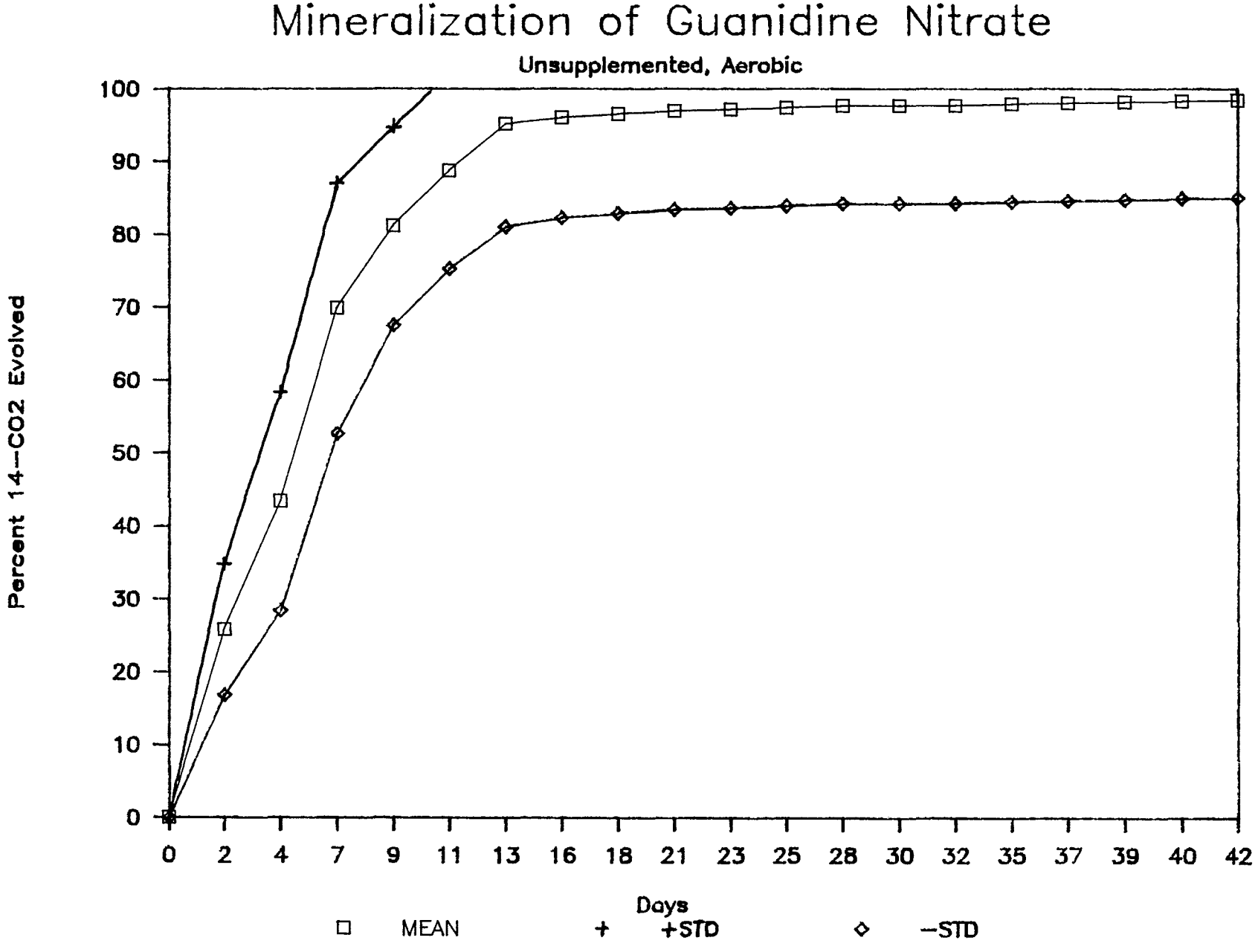

MINERALIZATION RATE OF NQ IN SFAAP SOIL
WHEY 100 MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

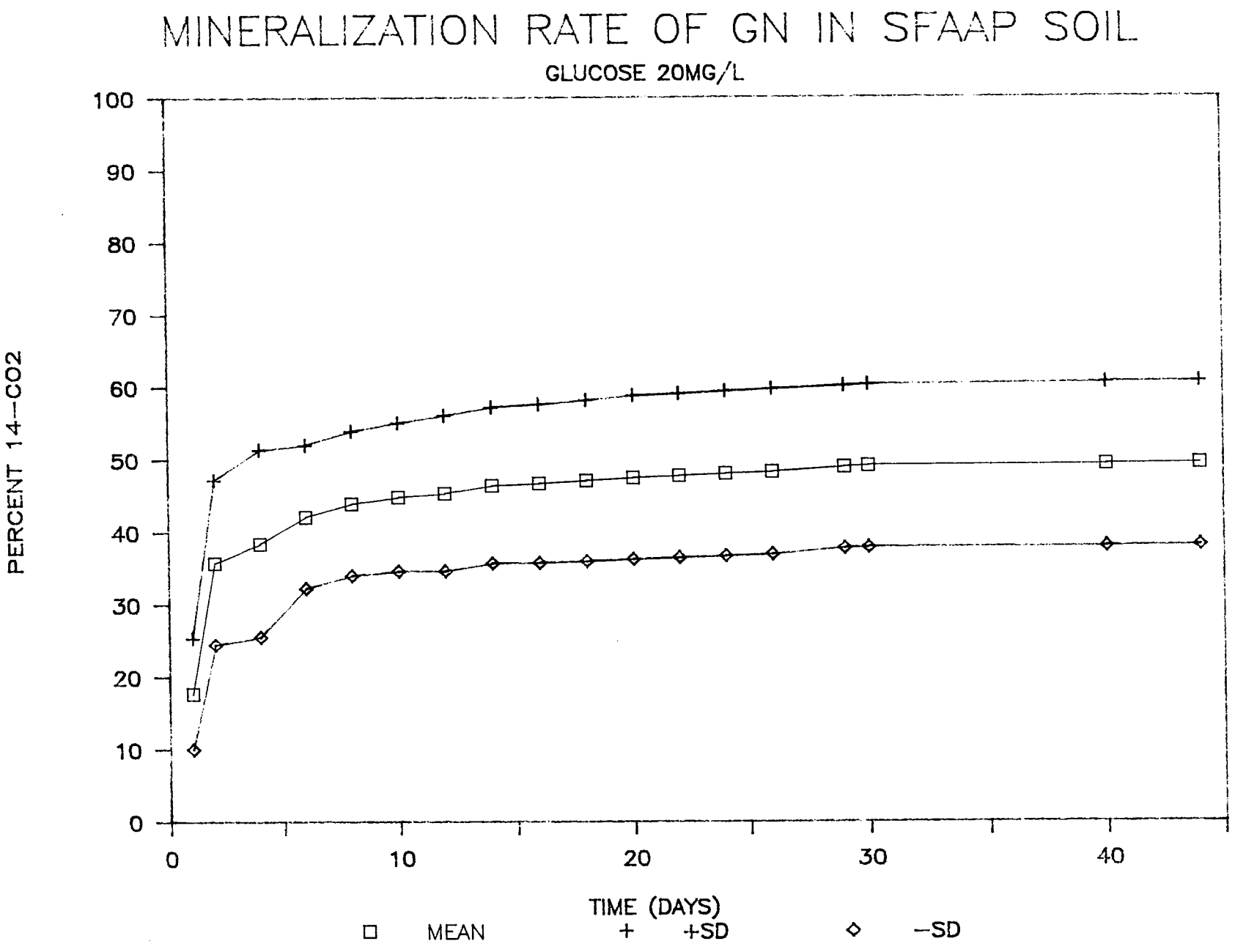

MINERALIZATION RATE OF NQ IN SFAAP SOIL
WHEY 200 MG/L
PERCENT 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
--SD

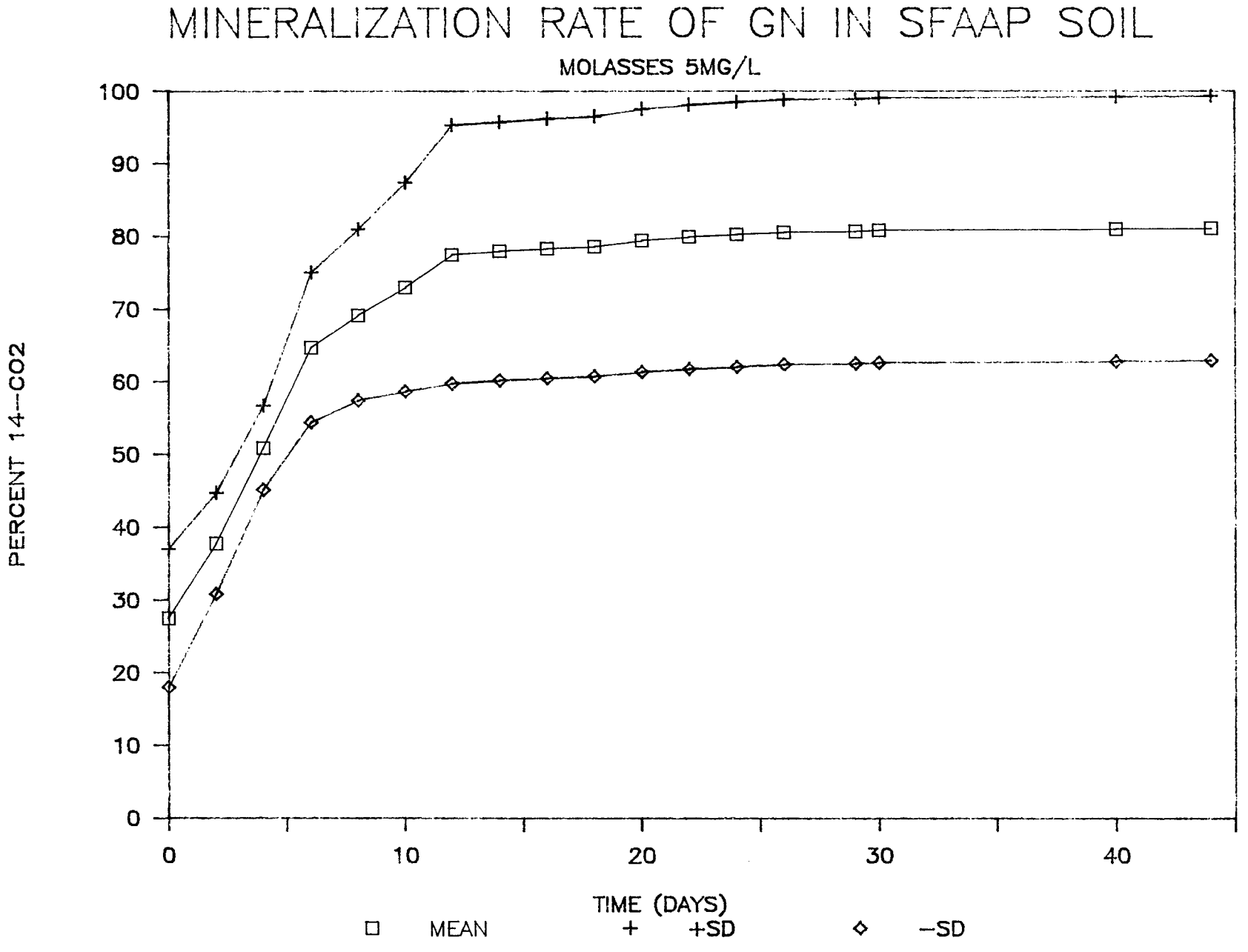
# MINERALIZATION RATE OF GN IN SFAAP SOIL

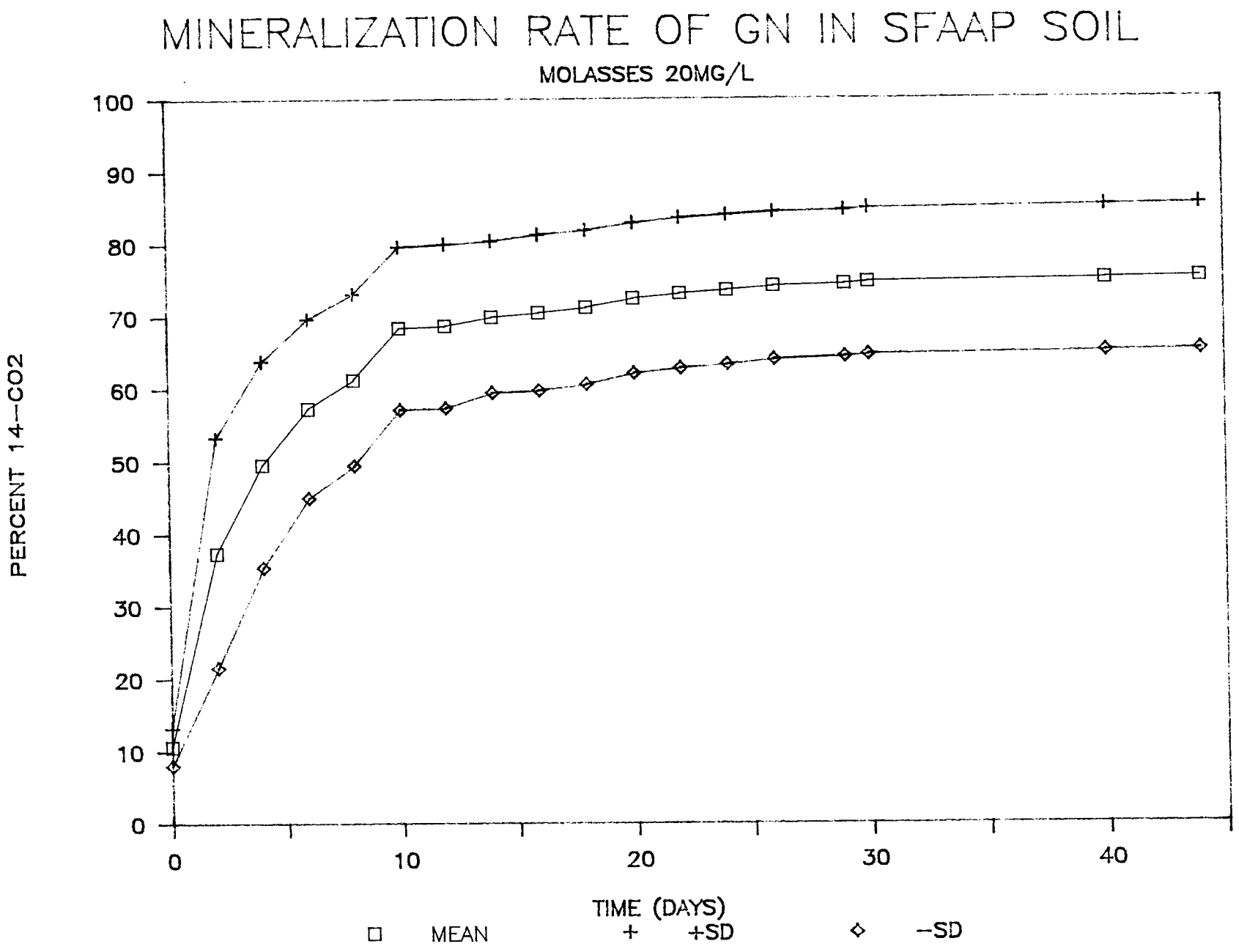
STERILE AUTOCLAVE

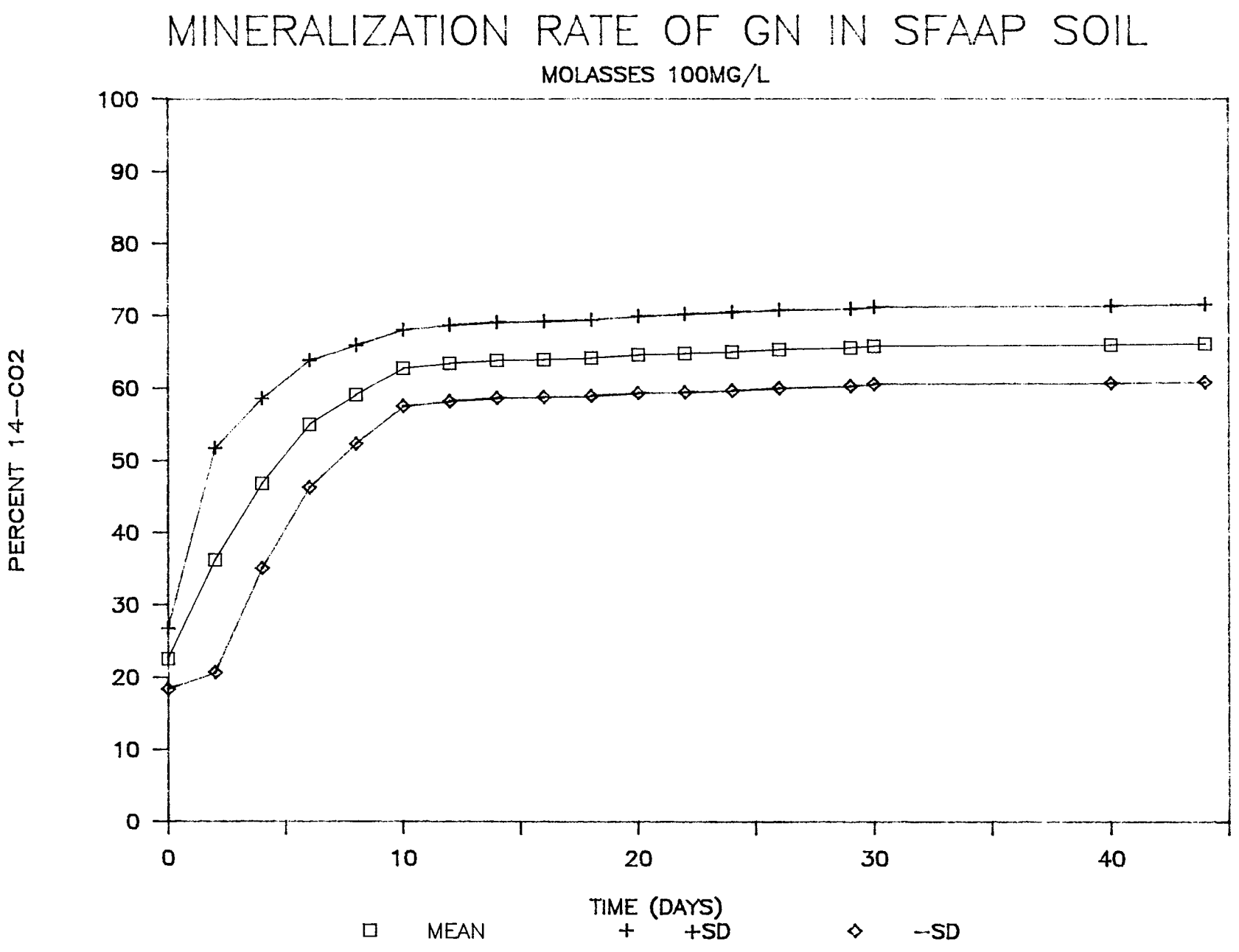



Mineralization of Guanidine Nitrate
Unsupplemented, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0 2 4 7 9 11 13 16 18 21 23 25 28 30 32 35 37 39 40 42
Days
MEAN
+STD
-STD

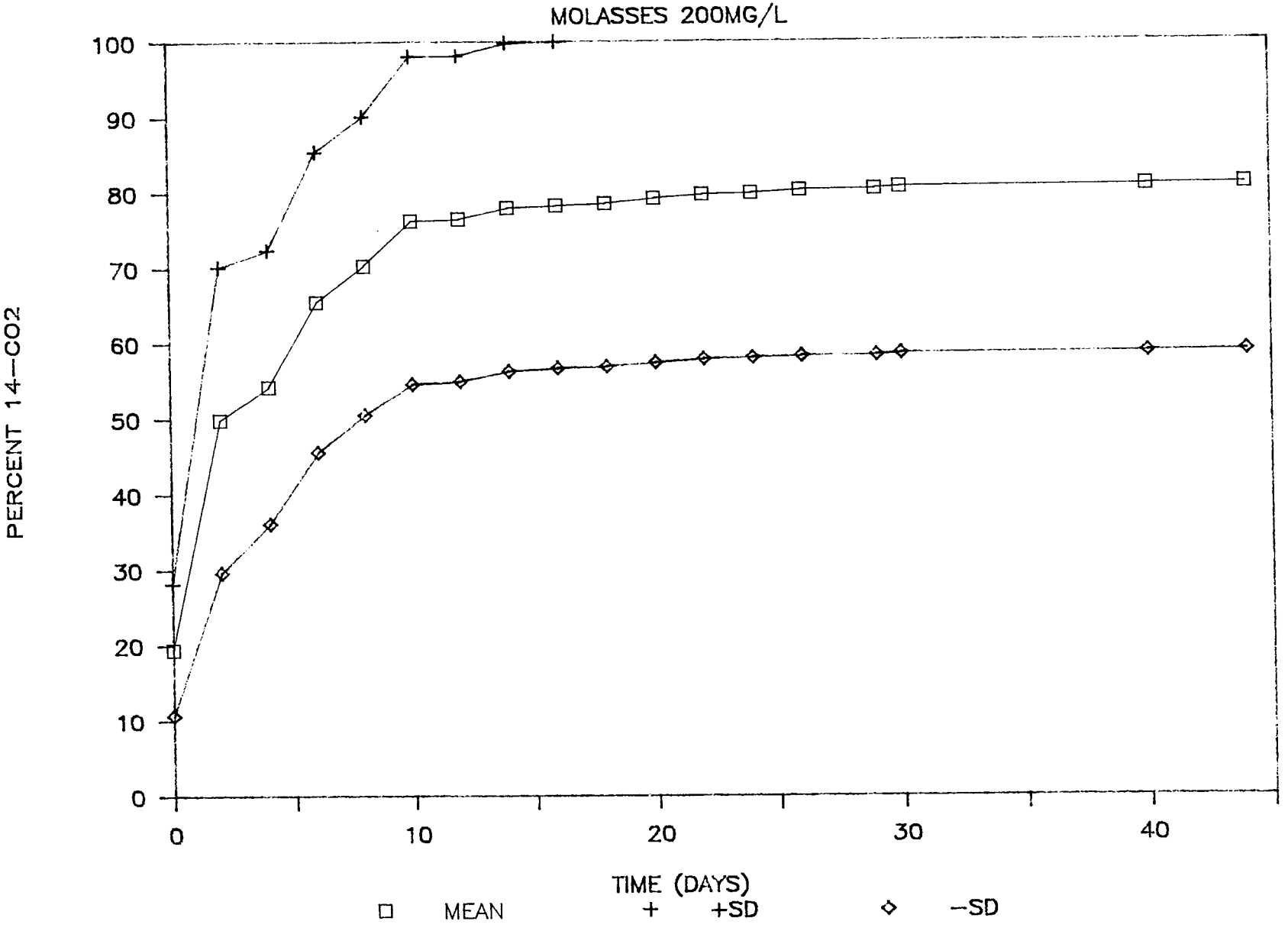

MINERALIZATION RATE OF GN IN SFAAP SOIL
GLUCOSE 20MG/L
PERCENT 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
—SD

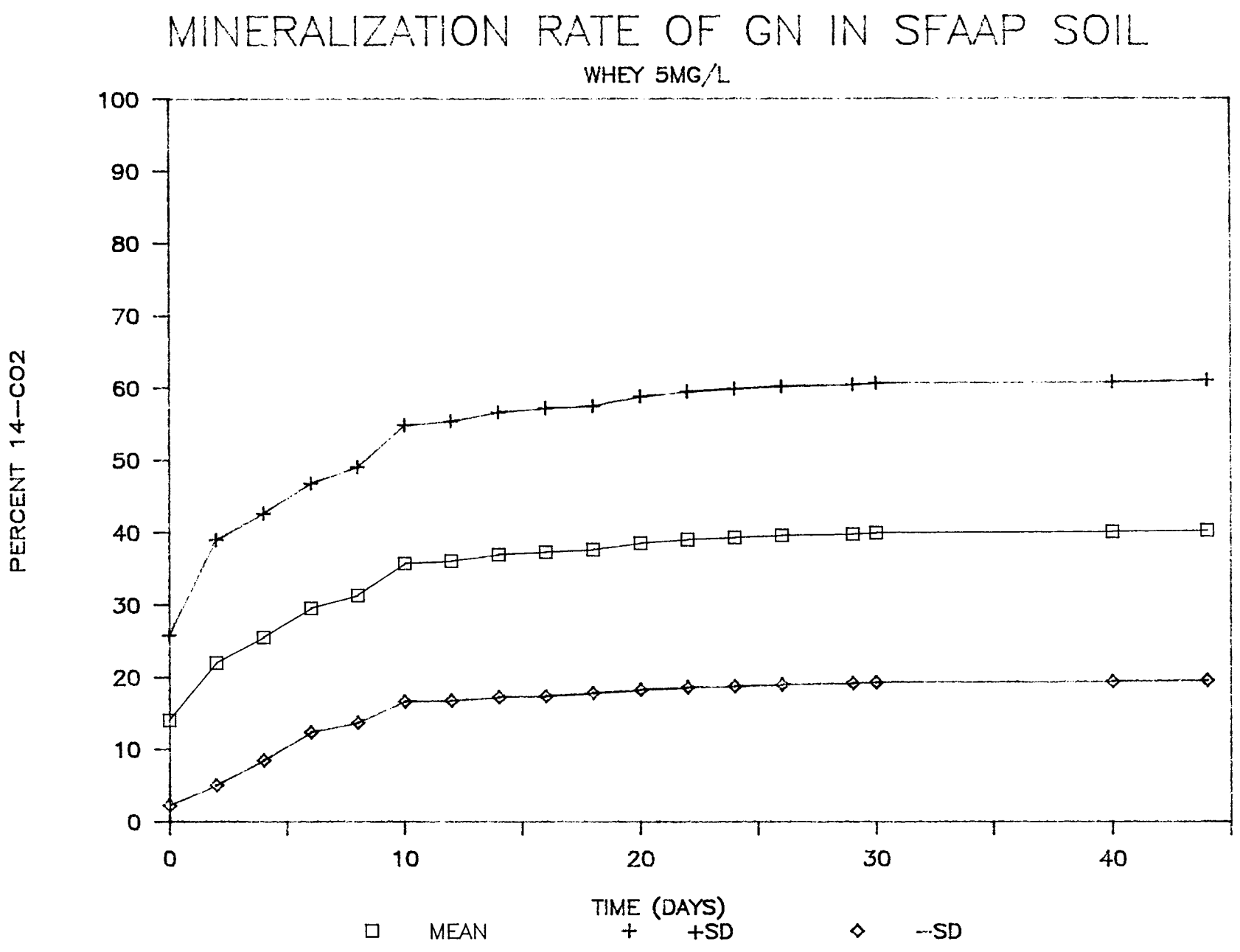

MINERALIZATION RATE OF GN IN SFAAP SOIL
MOLASSES 5MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

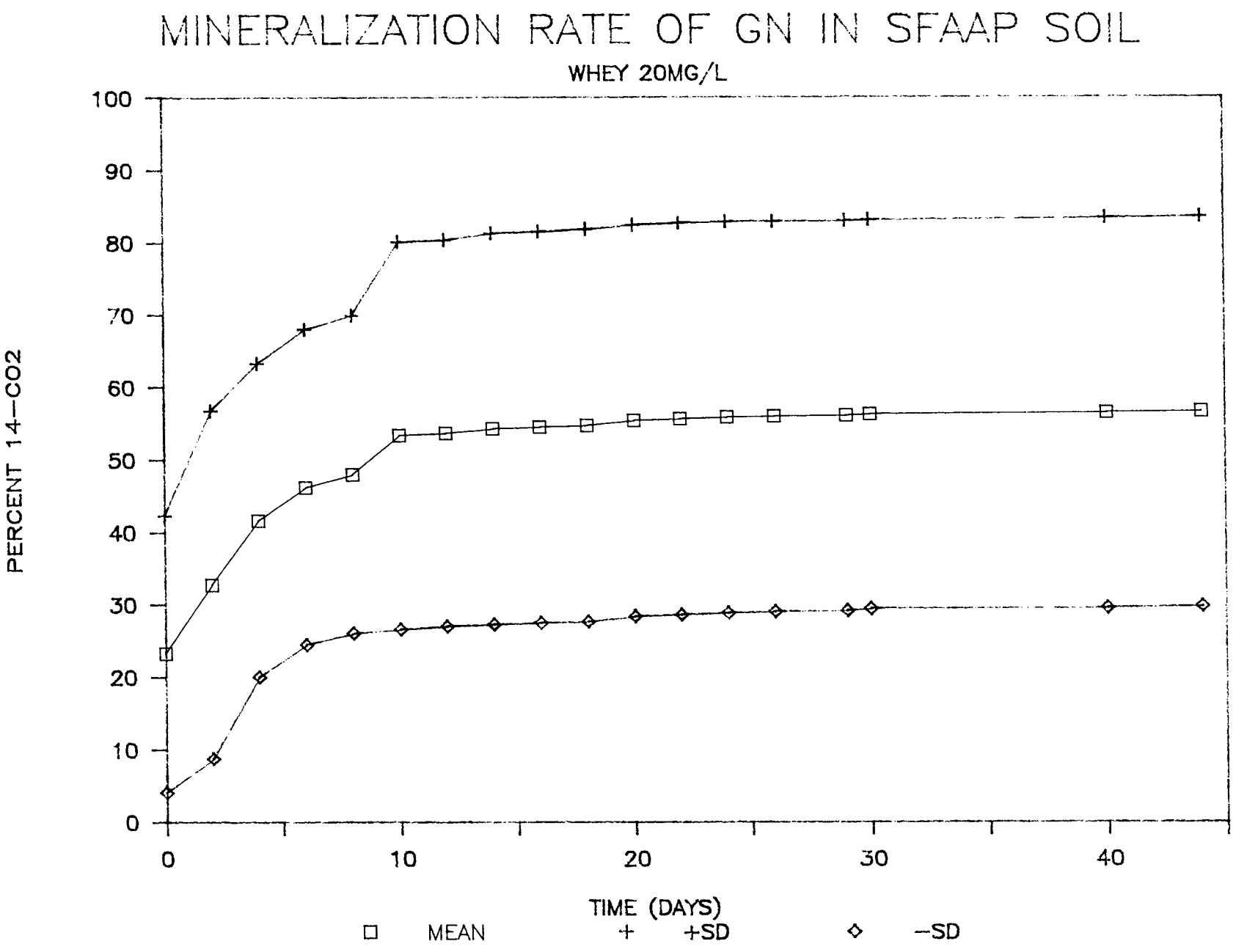

MINERALIZATION RATE OF GN IN SFAAP SOIL
MOLASSES 20MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD

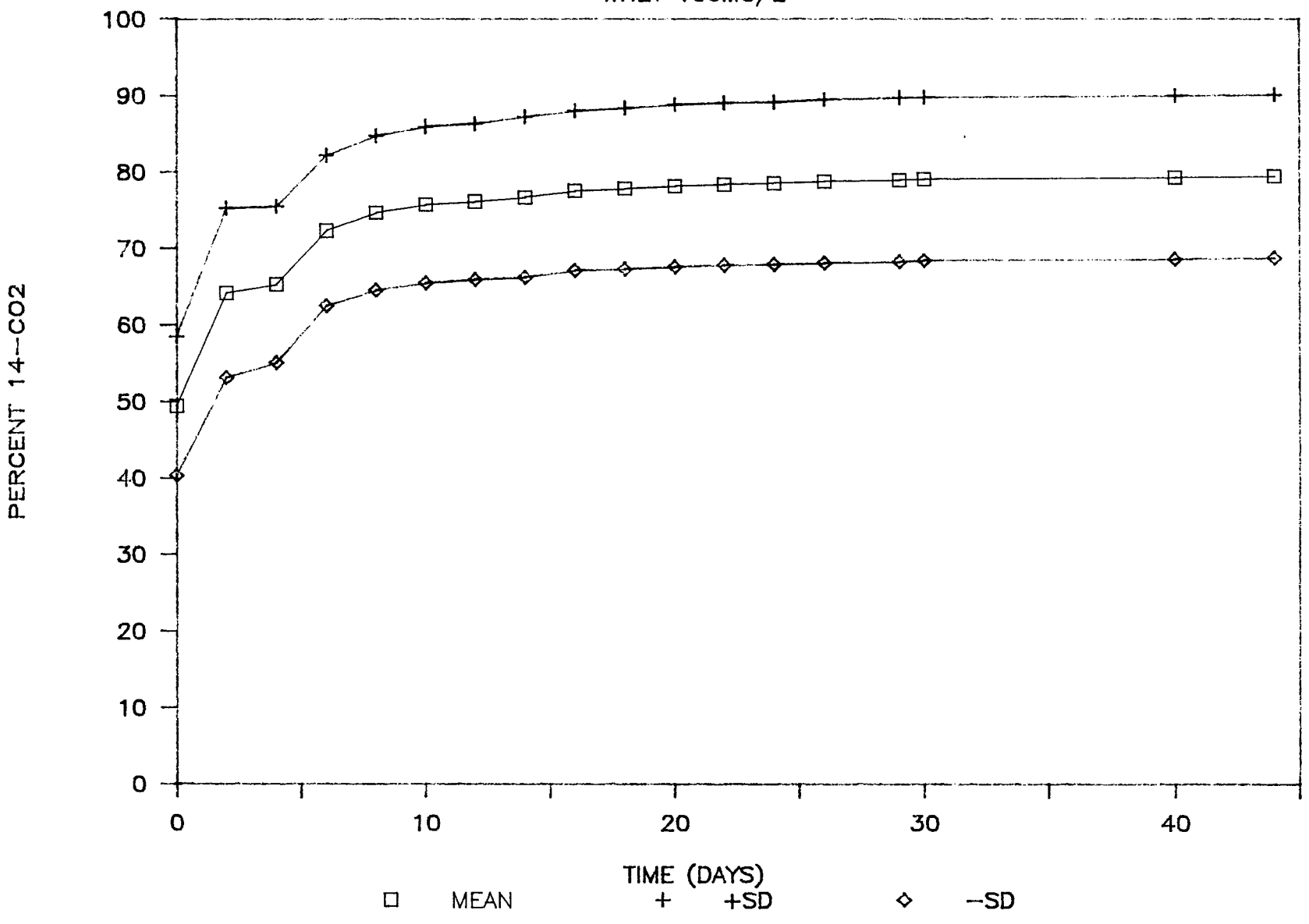

MINERALIZATION RATE OF GN IN SFAAP SOIL
MOLASSES 100MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
--SD

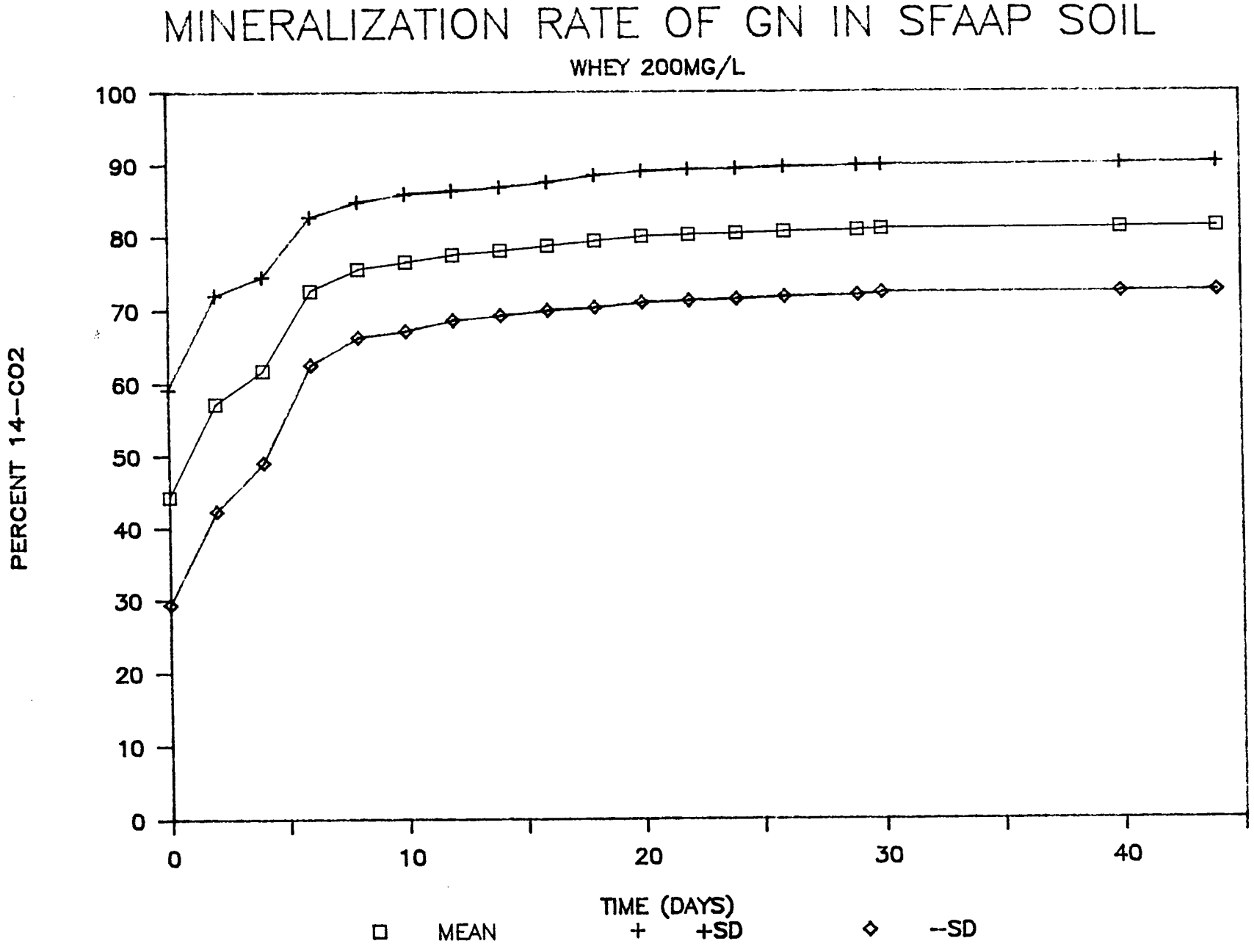
# MINERALIZATION RATE OF GN IN SFAAP SOIL




MINERALIZATION RATE OF GN IN SFAAP SOIL
WHEY 5MG/L
PERCENT 14--CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
--SD

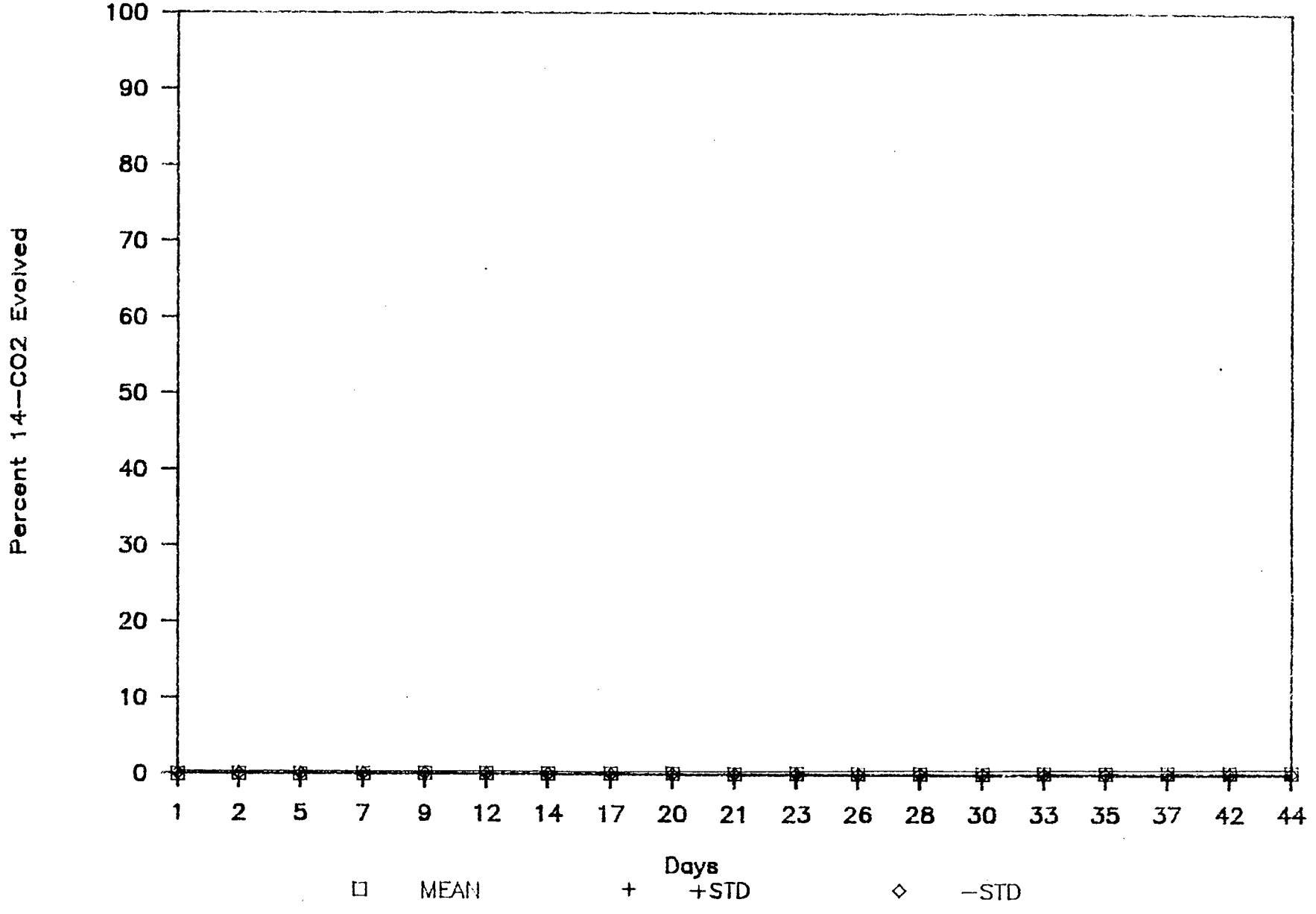

MINERALIZATION RATE OF GN IN SFAAP SOIL
WHEY 20MG/L
PERCENT 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
—SD


MINERALIZATION RATE OF GN IN SFAAP SOIL
WHEY 100MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
-SD


MINERALIZATION RATE OF GN IN SFAAP SOIL
WHEY 200MG/L
PERCENT 14-CO2
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (DAYS)
MEAN
+SD
--SD

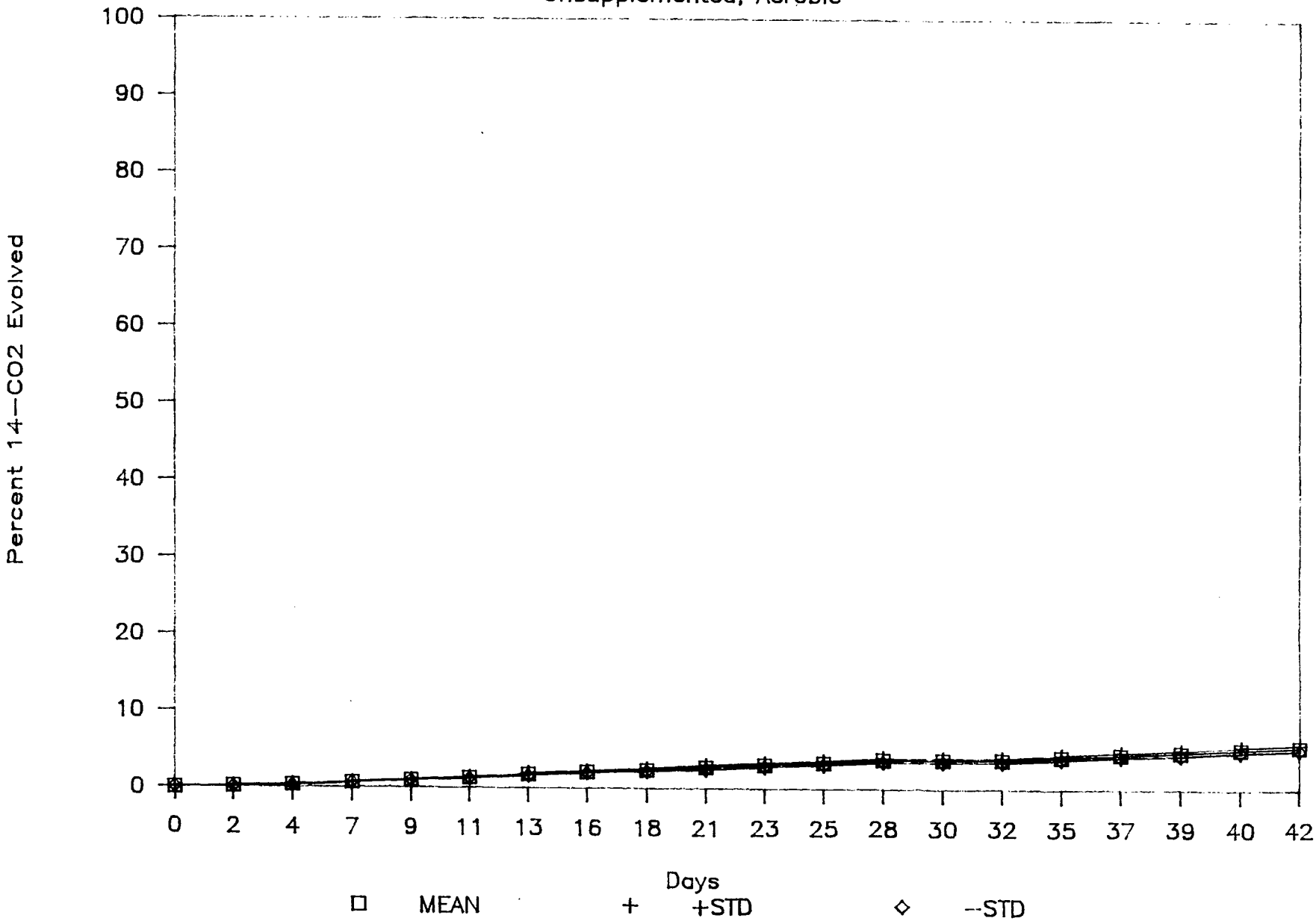
APPENDIX K

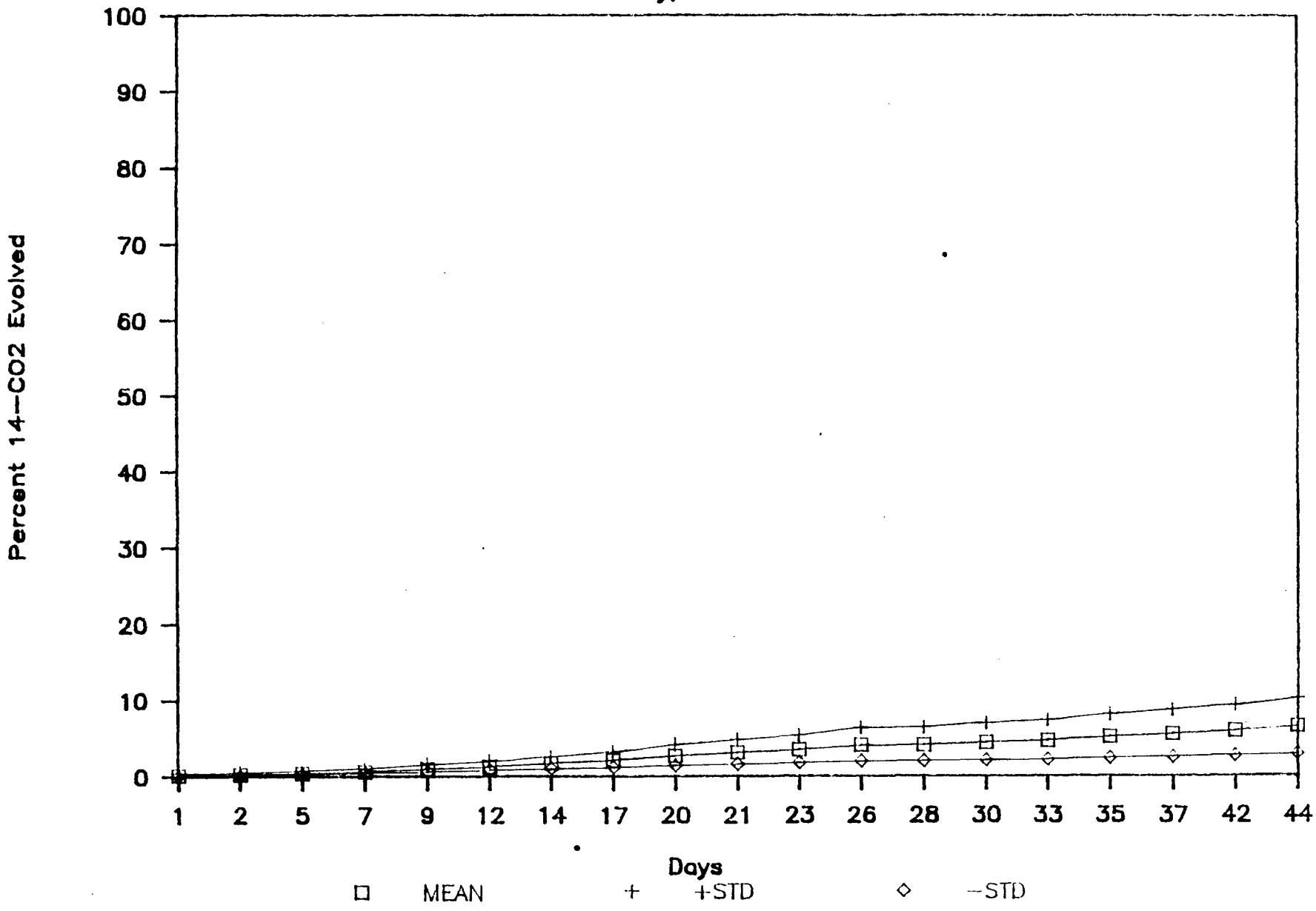
GRAPHS OF NITROGUANIDINE MINERALIZATION IN PRETREATMENT SOIL - AEROBIC AND ANAEROBIC CONDITIONS


Mineralization of Nitroguanidine
Sterile, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
17
20
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
—STD

# Mineralization of Nitroguanidine

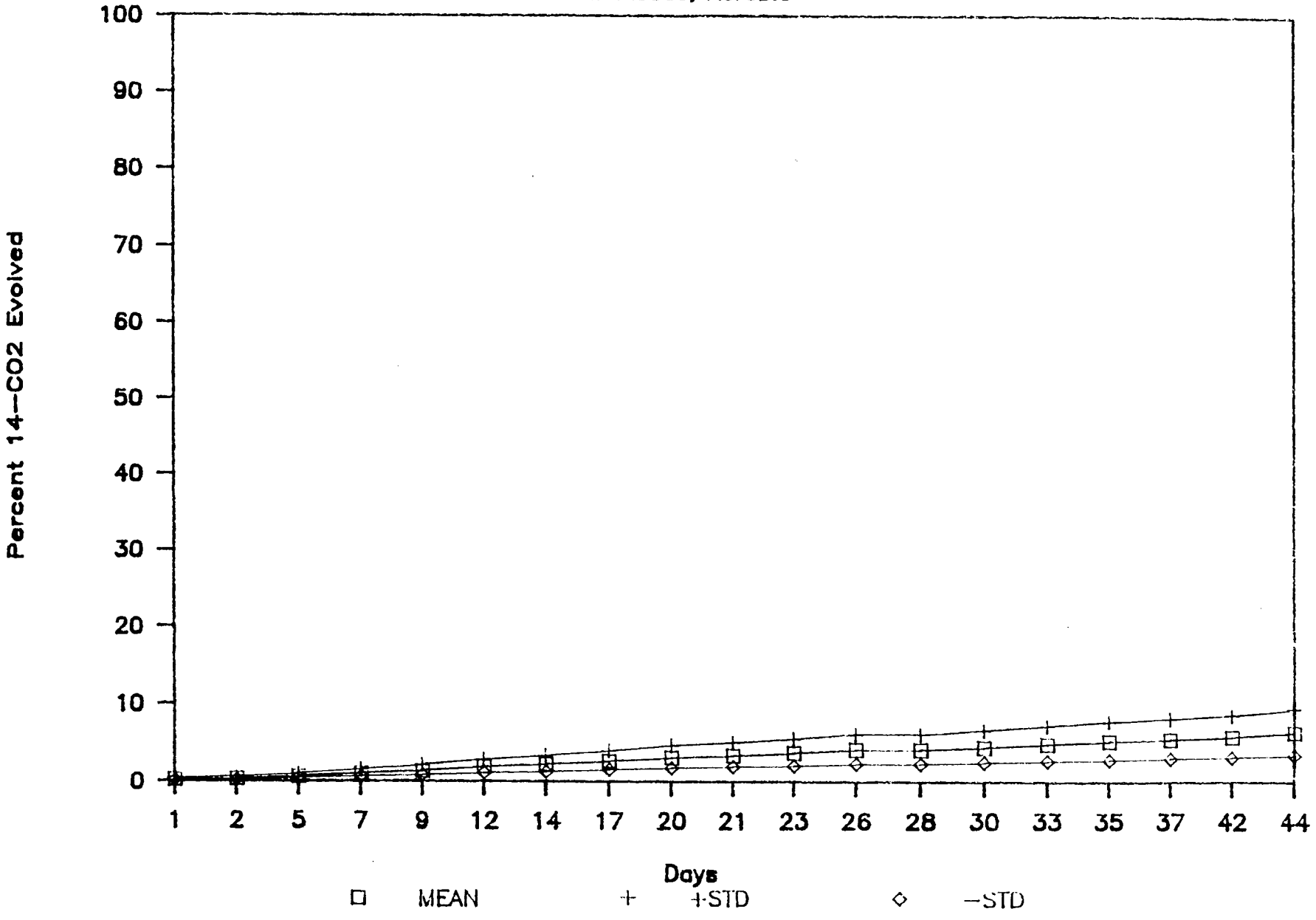
Unsupplemented, Aerobic

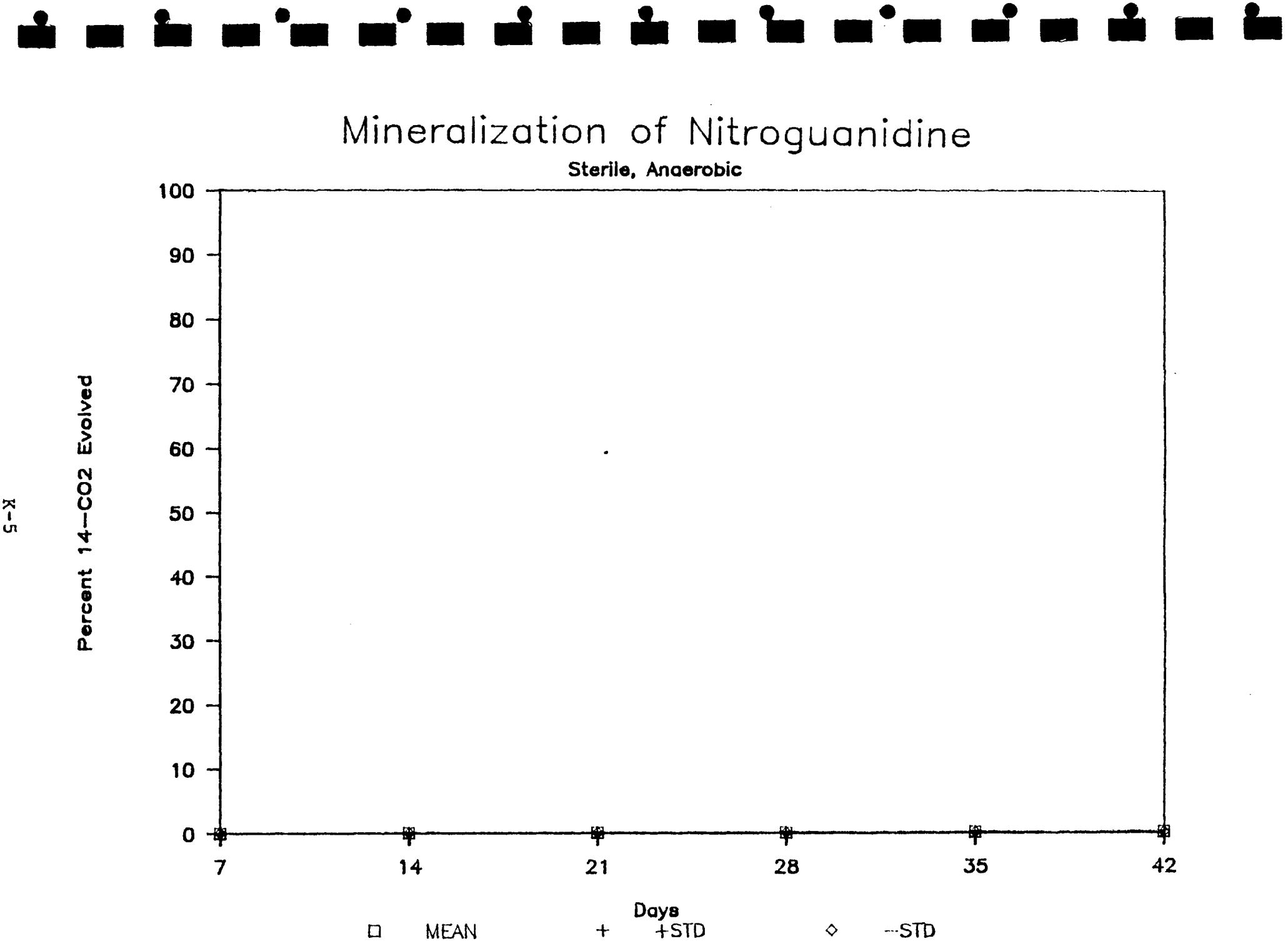



Mineralization of Nitroguanidine
Whey, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
17
20
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
—STD

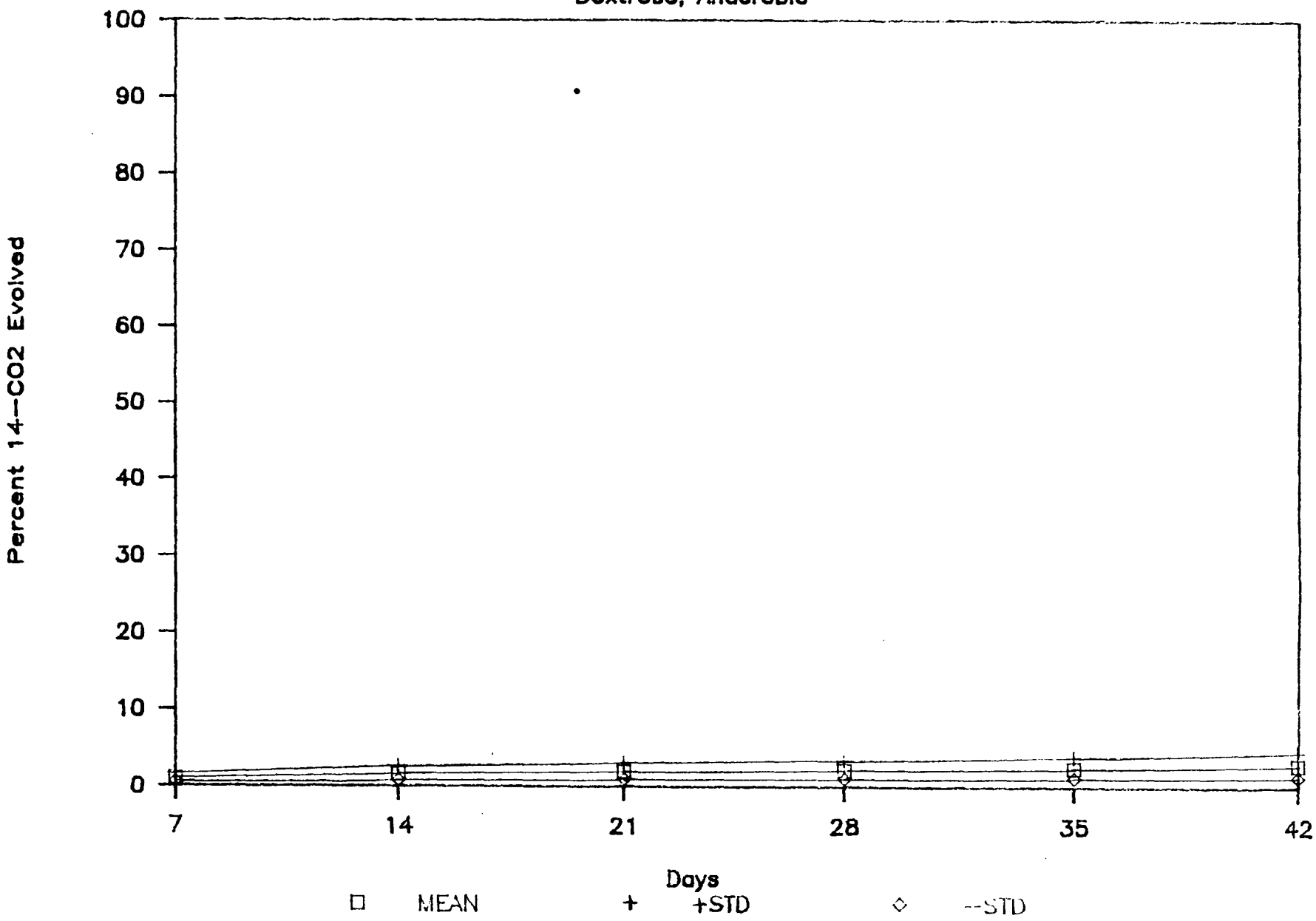
# Mineralization of Nitroguanidine

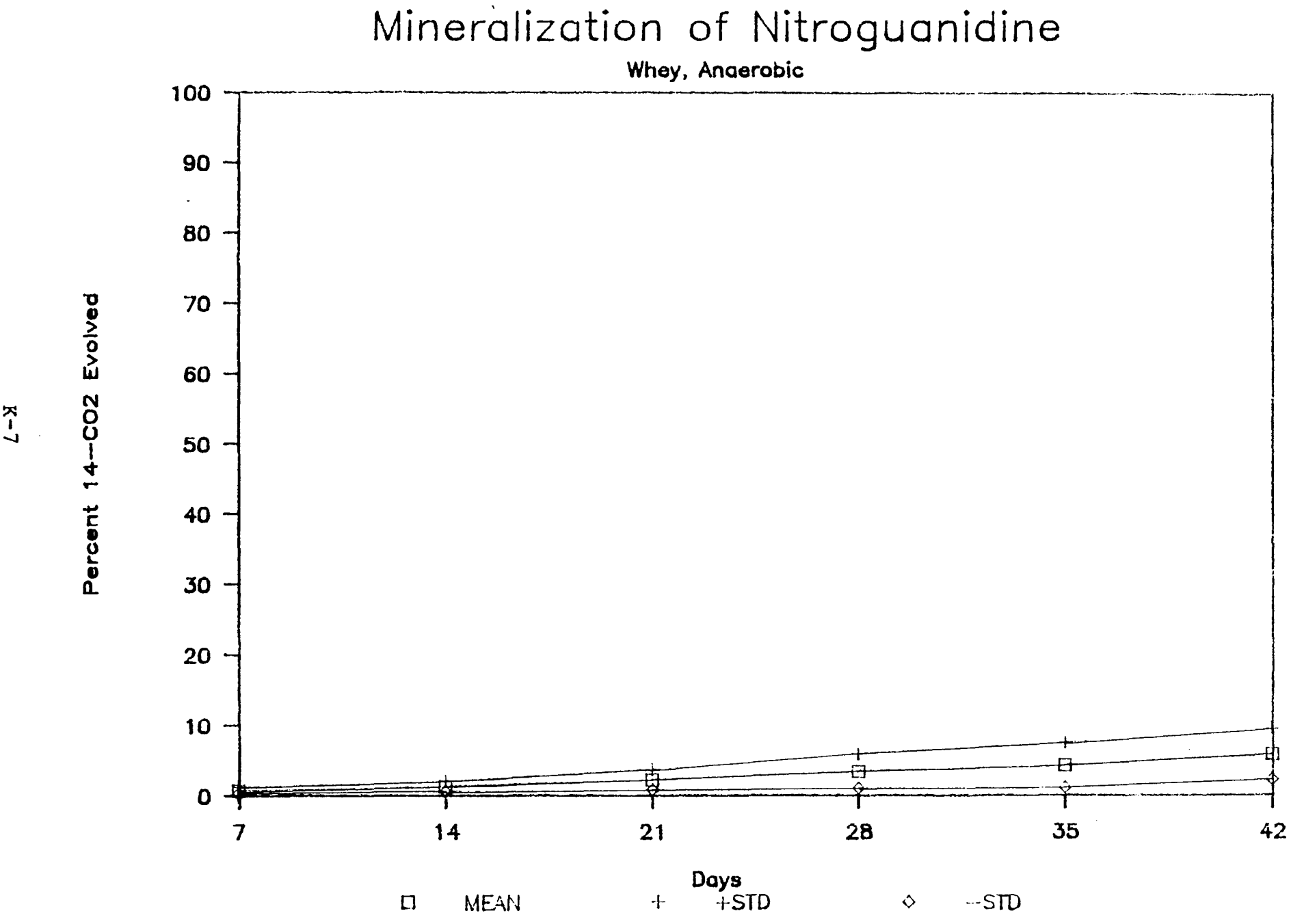
**Molasses, Aerobic**




Mineralization of Nitroguanidine
Sterile, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
—STD

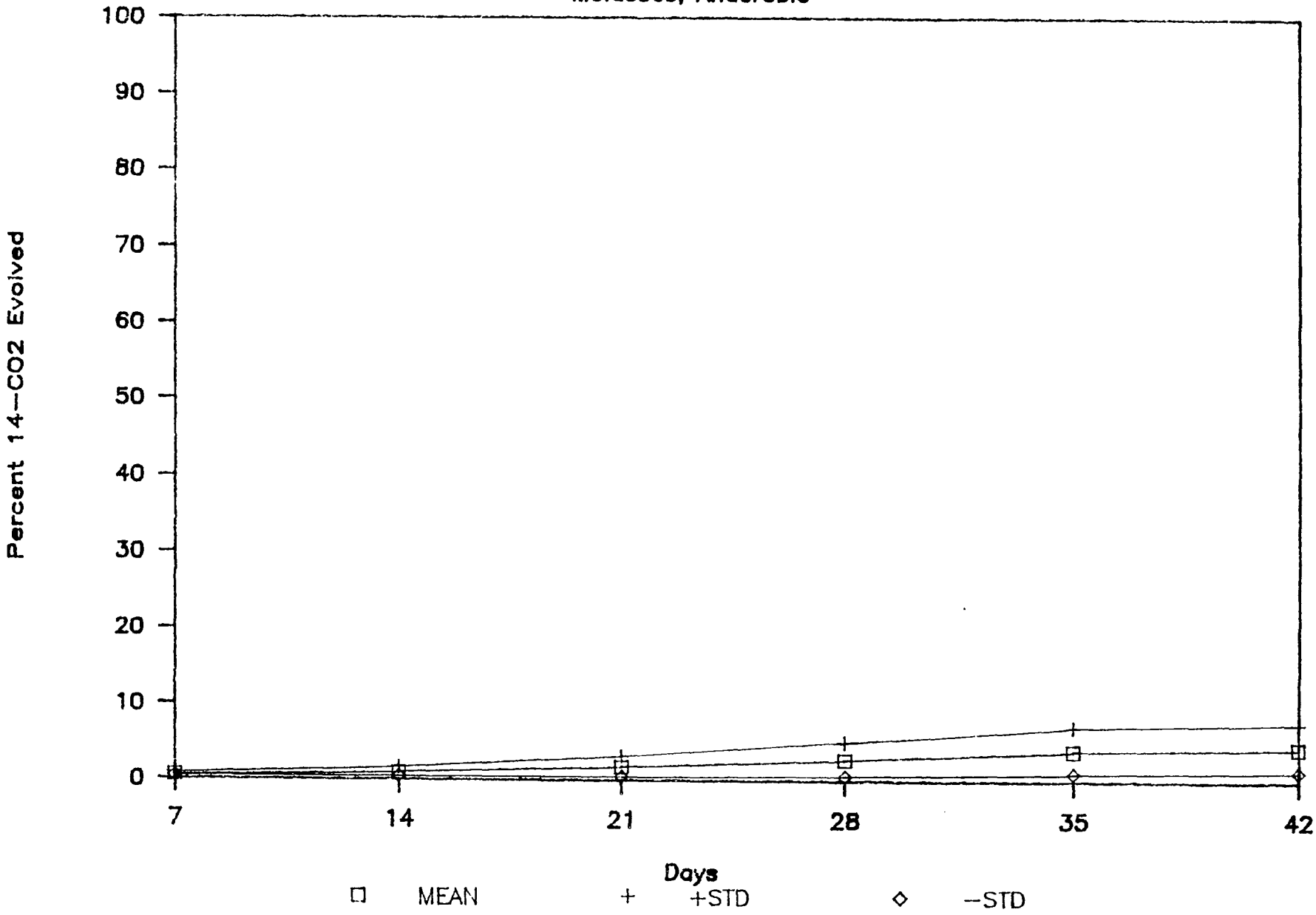
# Mineralization of Nitroguanidine




Mineralization of Nitroguanidine
Whey, Anaerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
--STD

# Mineralization of Nitroguanidine

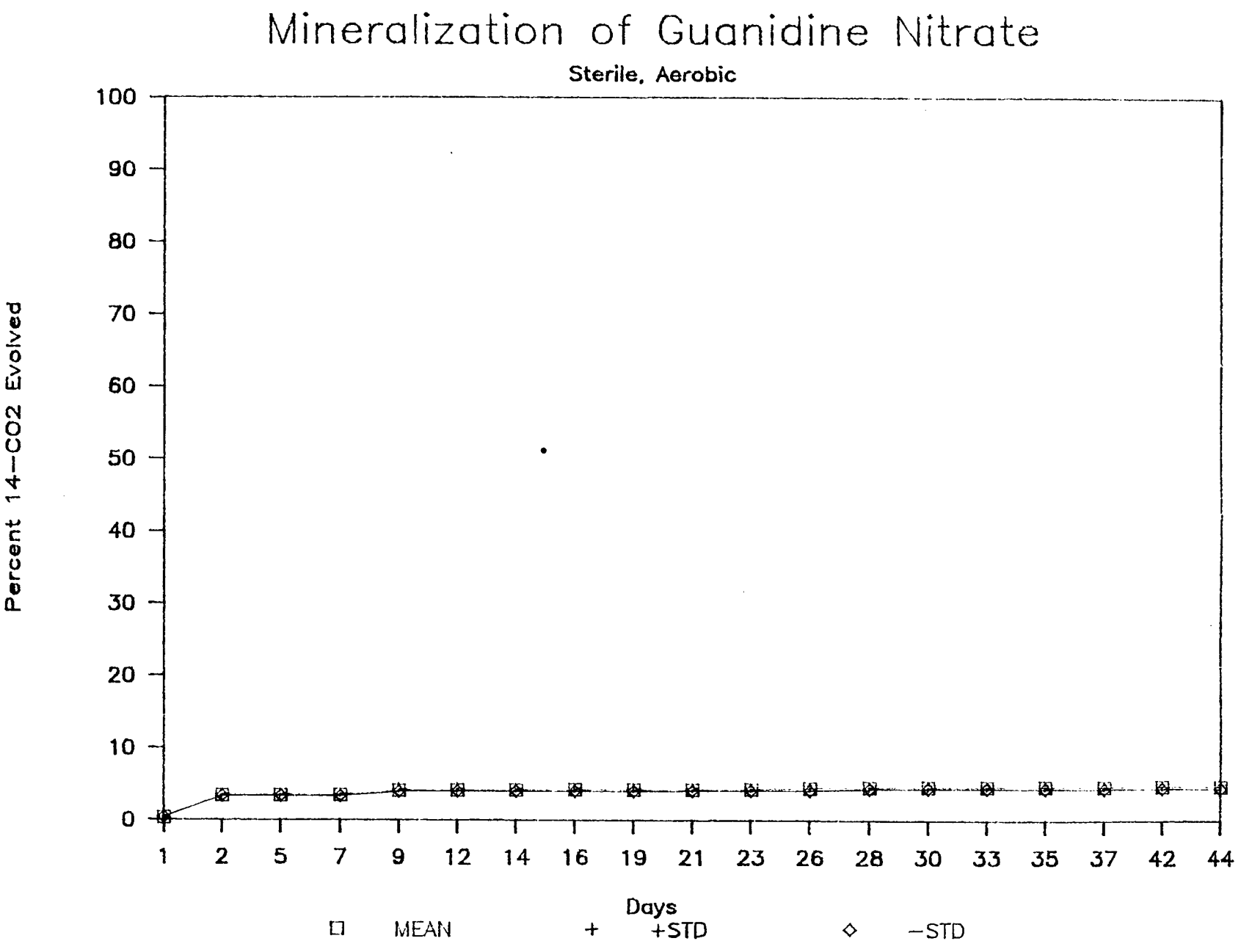
**Molasses, Anaerobic**



WESTON
MANAGERS DESIGNERS CONSULTANTS

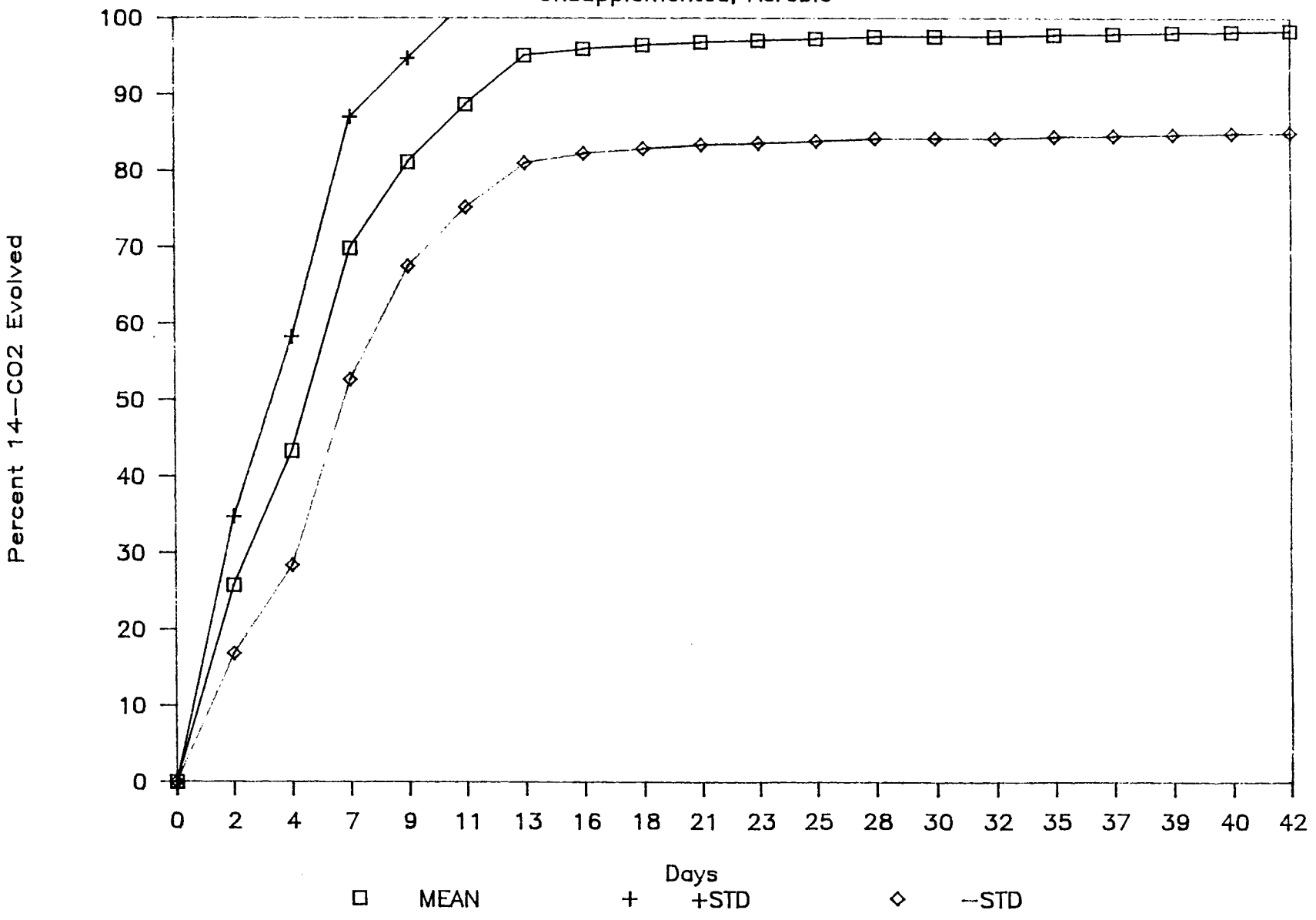
# APPENDIX L

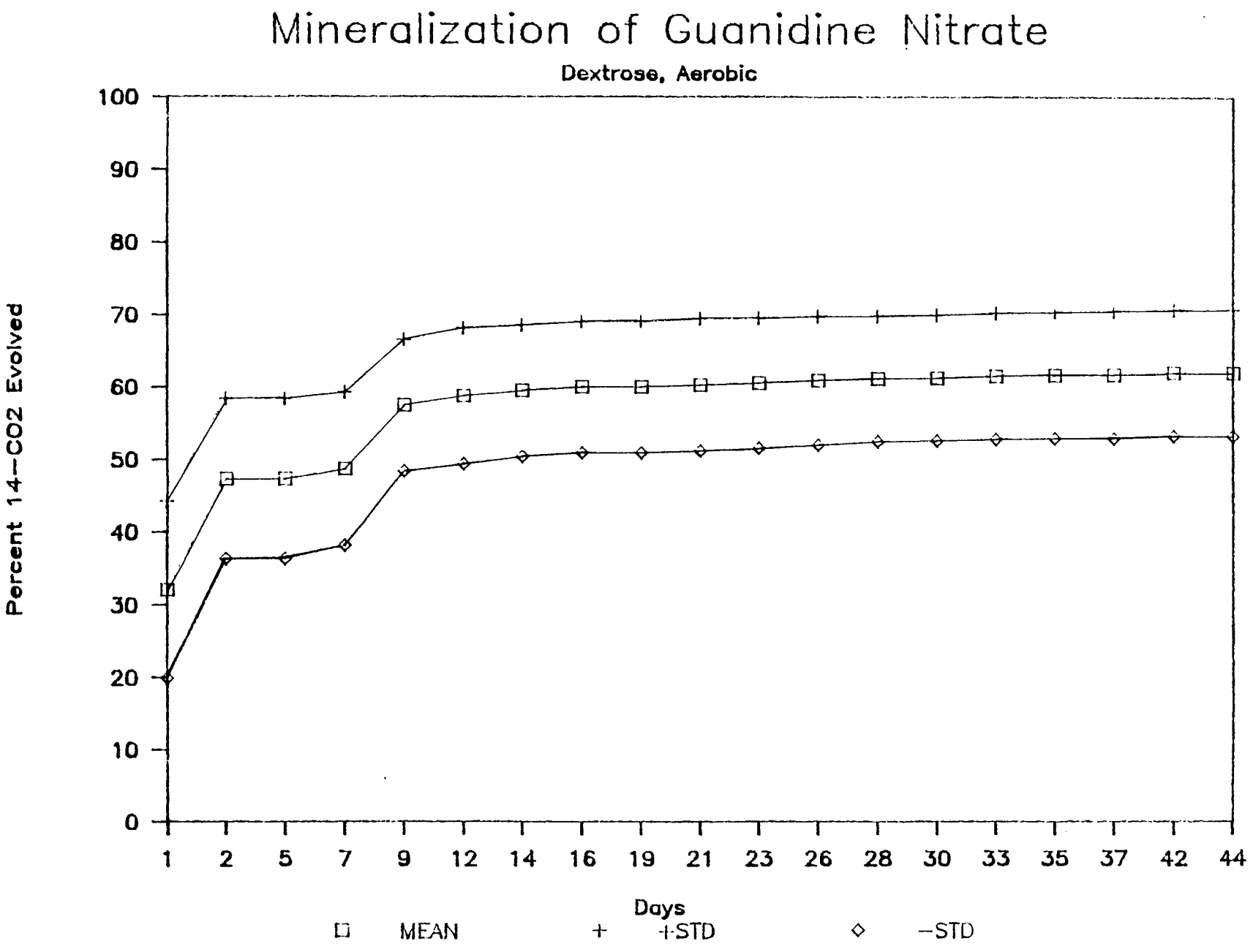
## GRAPHS OF GN MINERALIZATION IN PRETREATMENT SOIL - AEROBIC AND ANAEROBIC CONDITIONS


Mineralization of Guanidine Nitrate
Sterile, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
—STD

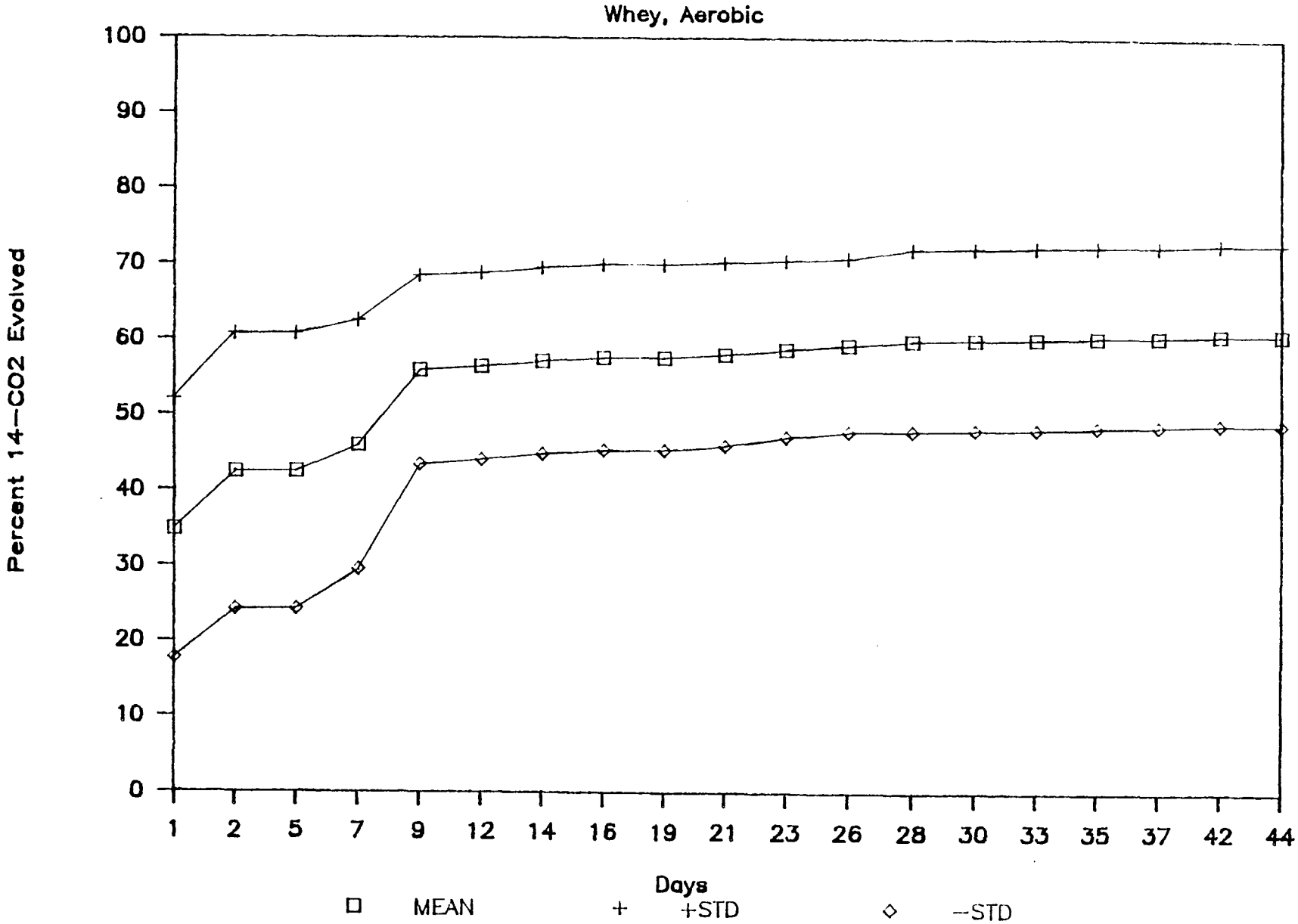
# Mineralization of Guanidine Nitrate

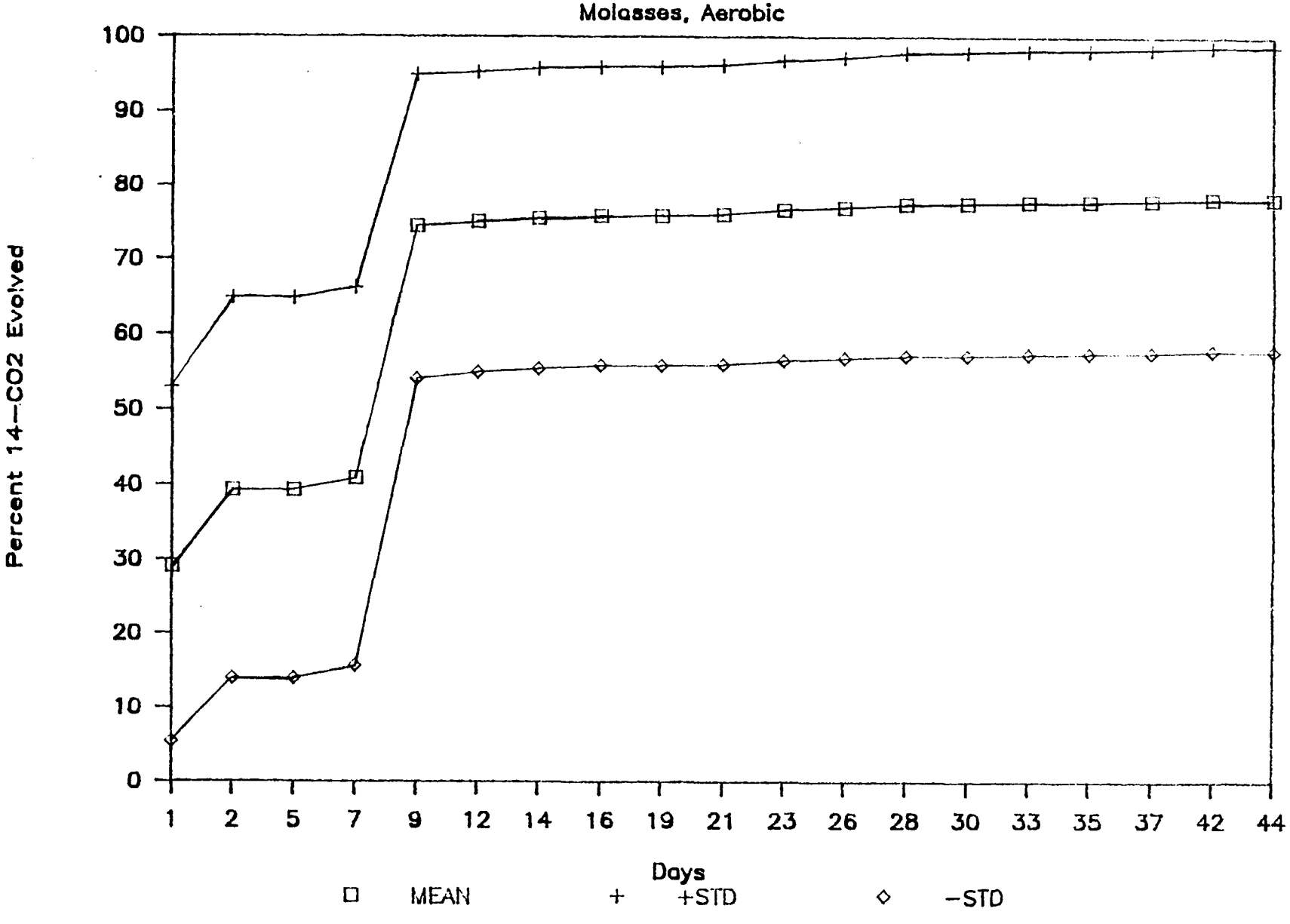
Unsupplemented, Aerobic



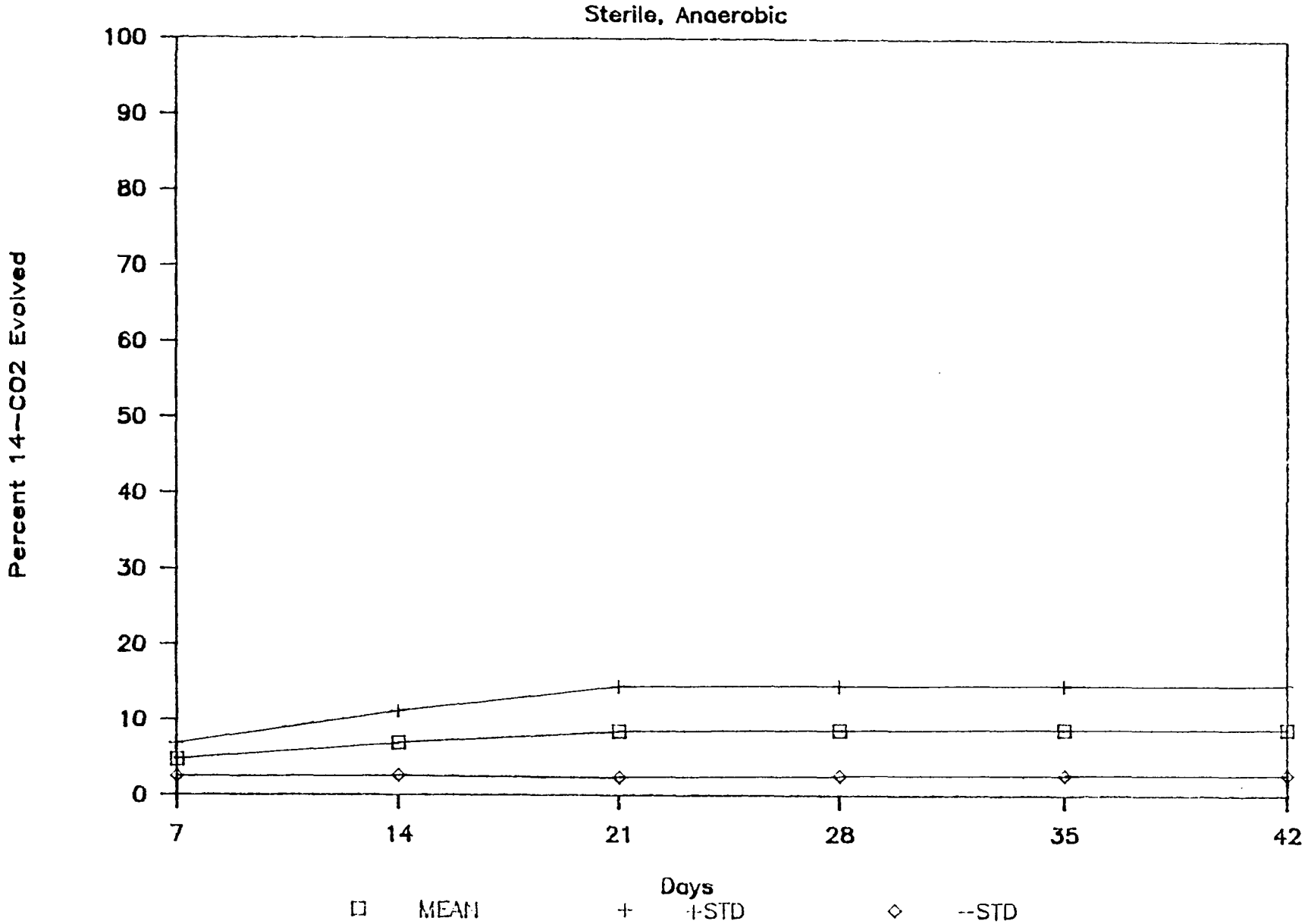

Mineralization of Guanidine Nitrate
Dextrose, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
-STD

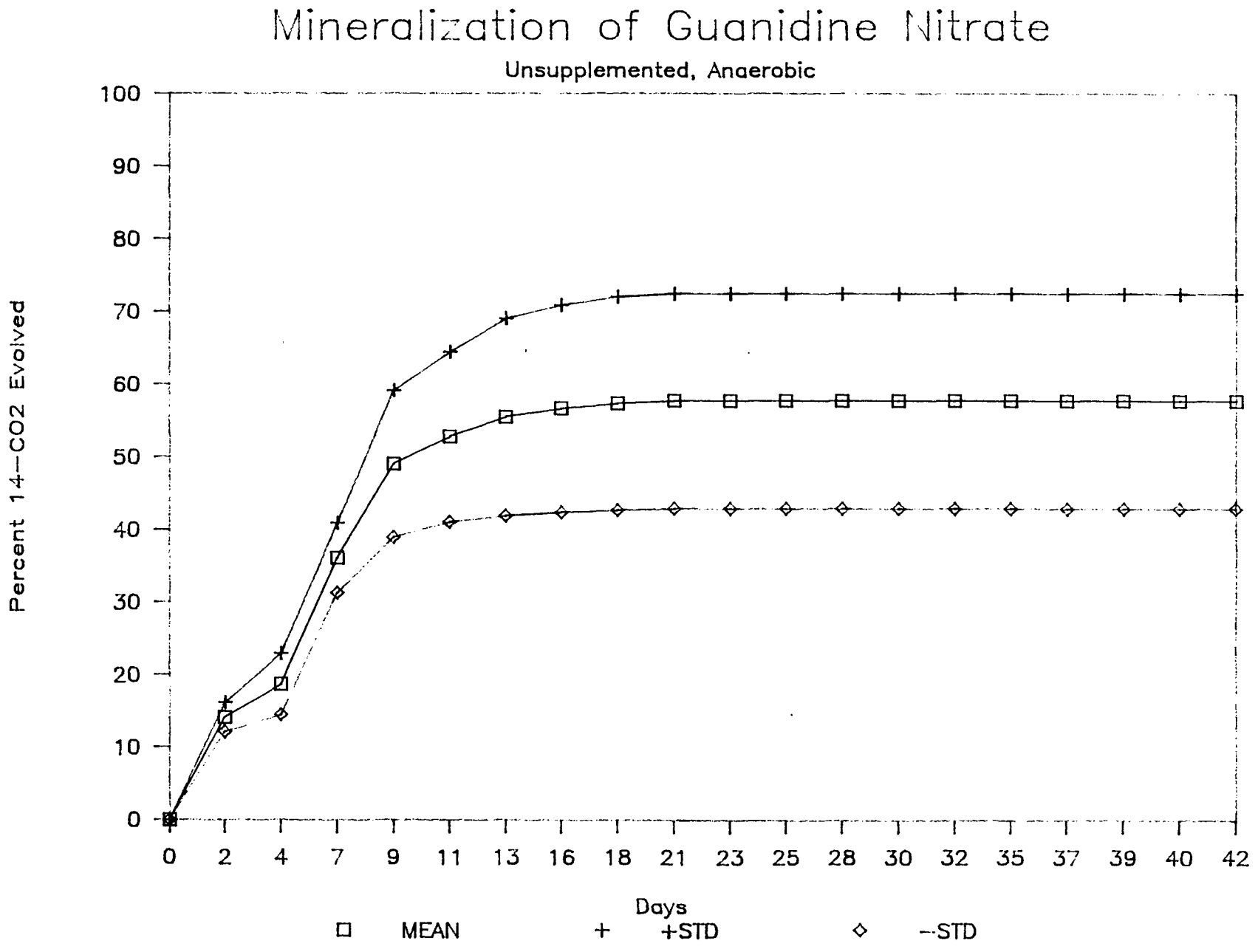
# Mineralization of Guanidine Nitrate



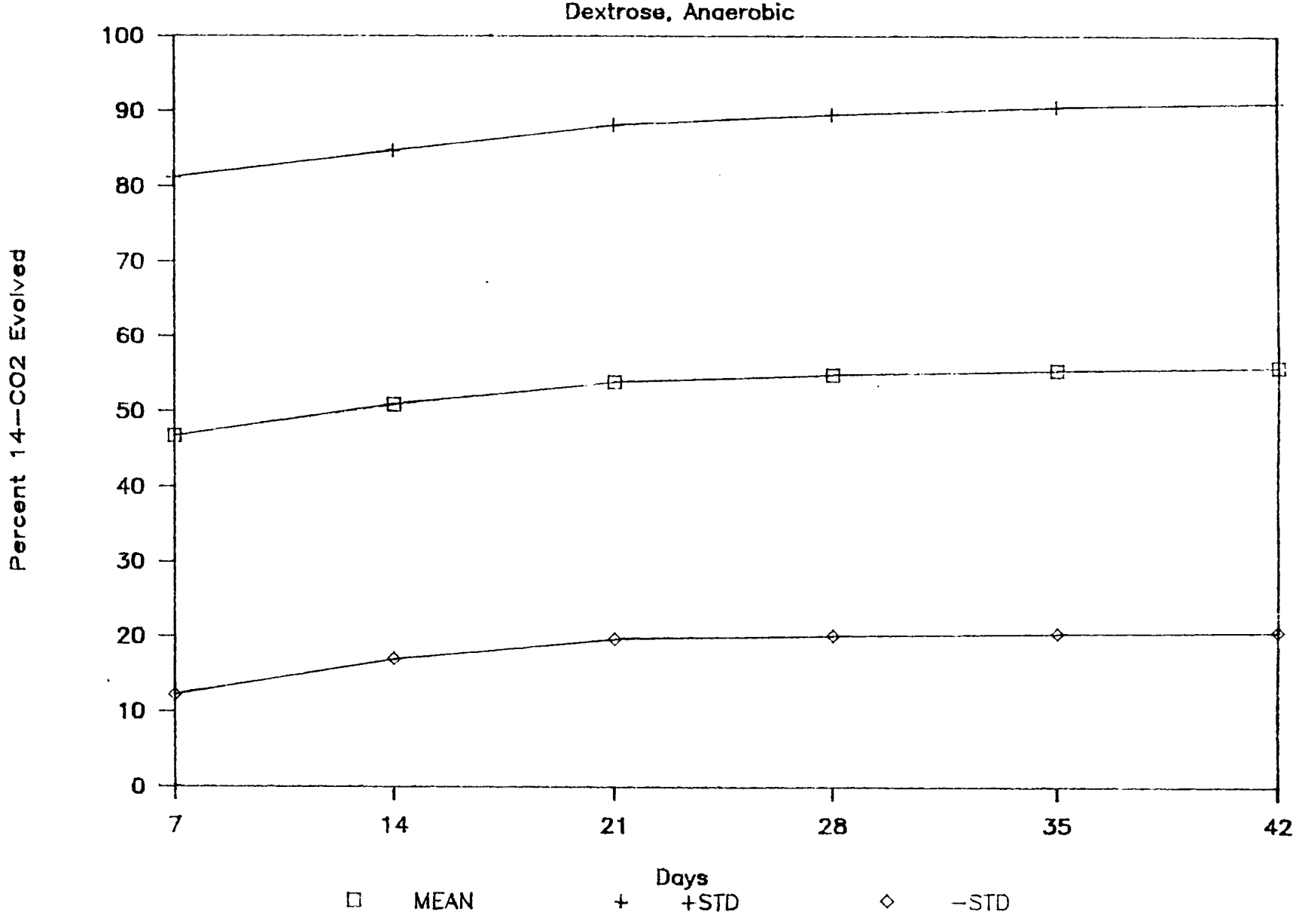

Mineralization of Guanidine Nitrate
Molasses, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
−STD

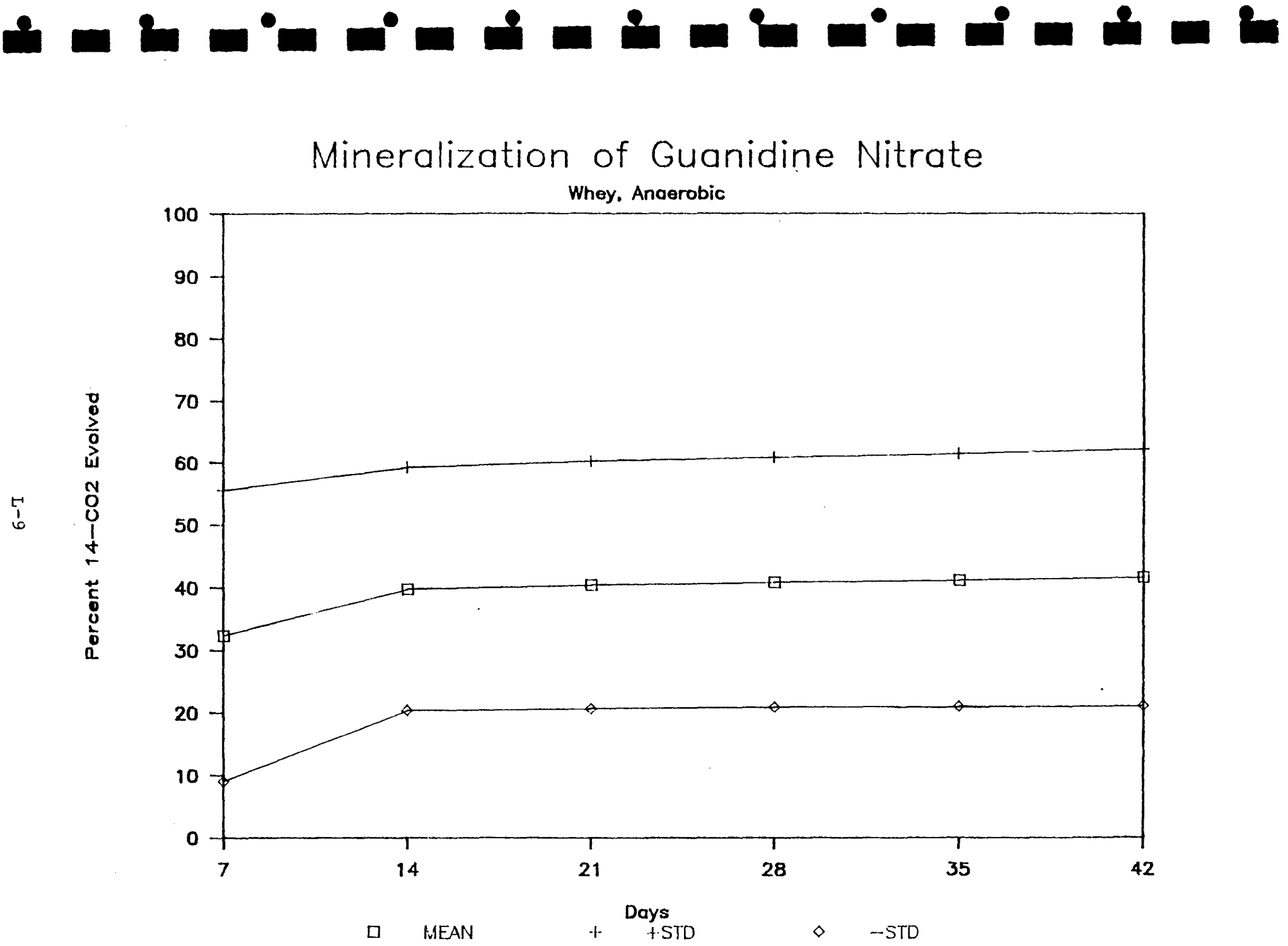
# Mineralization of Guanidine Nitrate



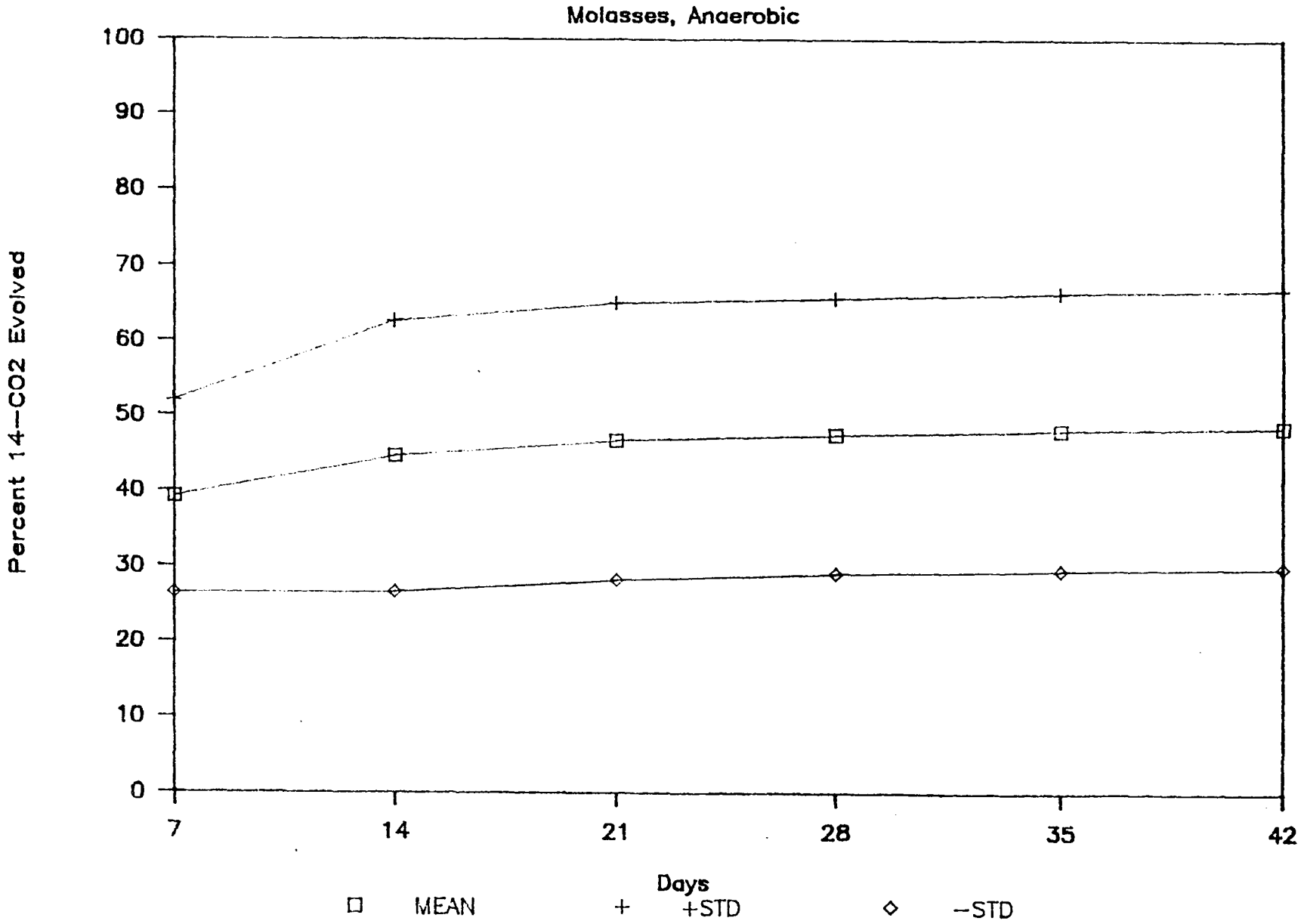

Mineralization of Guanidine Nitrate
Unsupplemented, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0 2 4 7 9 11 13 16 18 21 23 25 28 30 32 35 37 39 40 42
Days
MEAN
+STD
--STD

# Mineralization of Guanidine Nitrate




Mineralization of Guanidine Nitrate
Whey, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
—STD

# Mineralization of Guanidine Nitrate



# APPENDIX M

## GRAPHS OF GUANIDINE NITRATE VOLATILIZATION

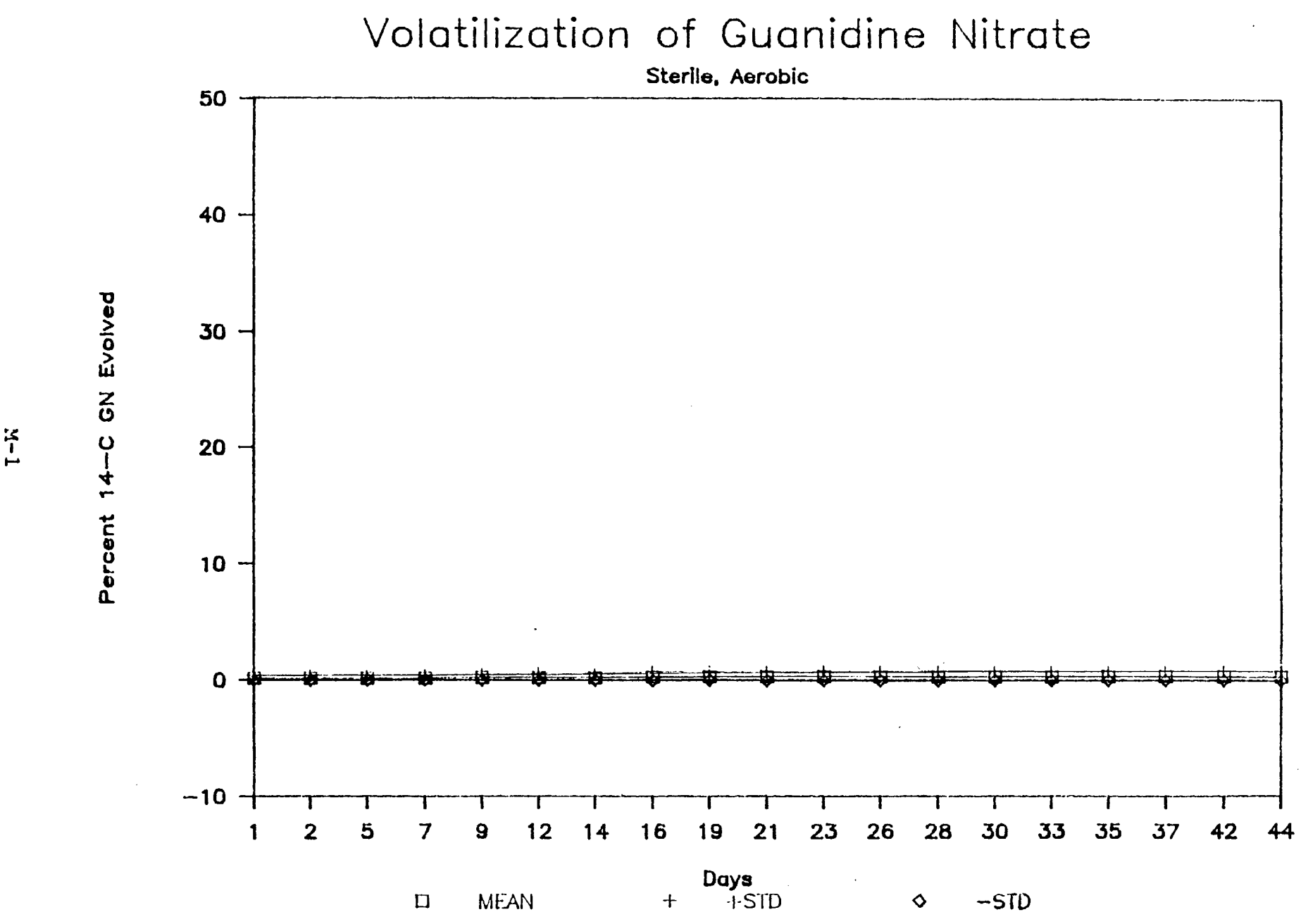
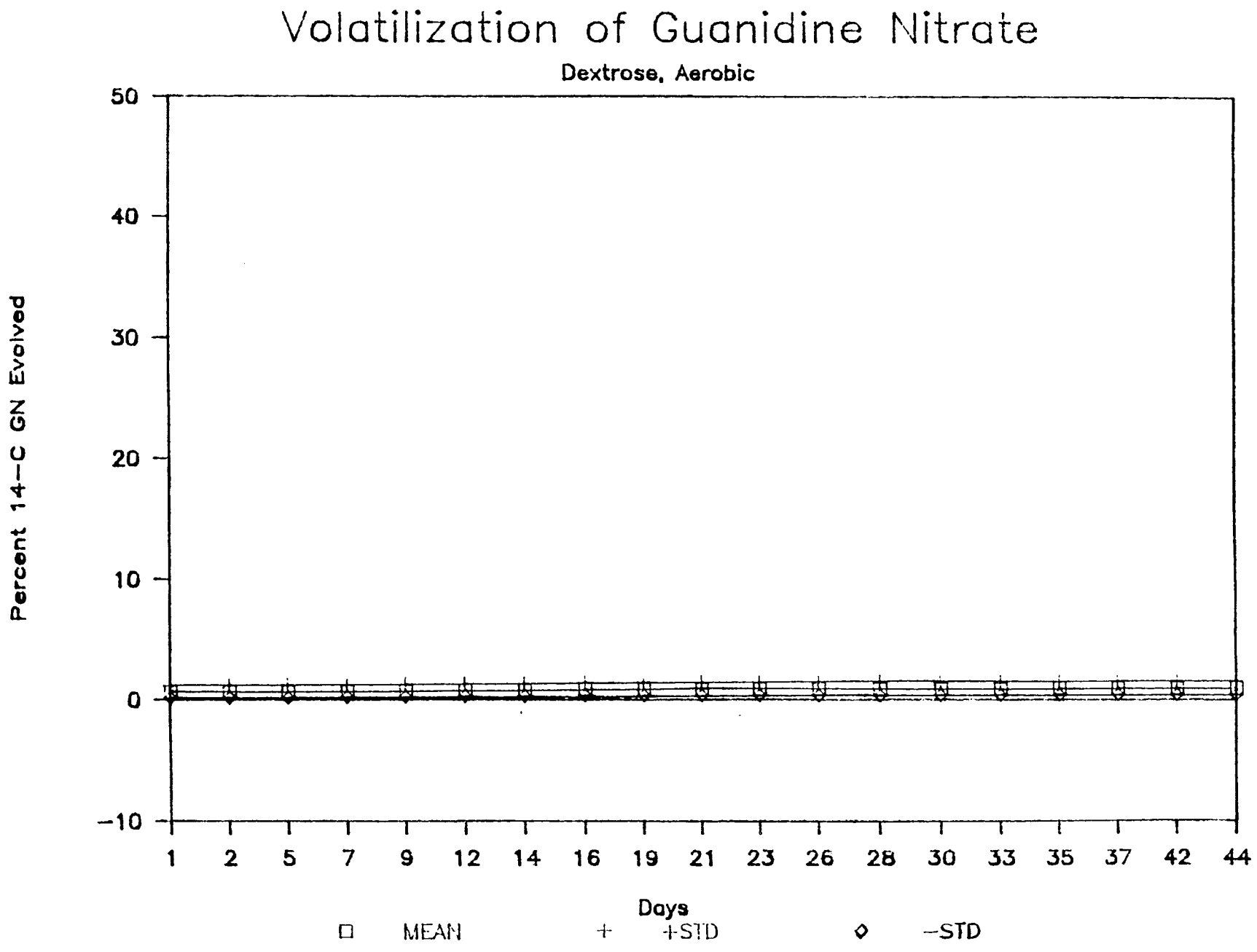
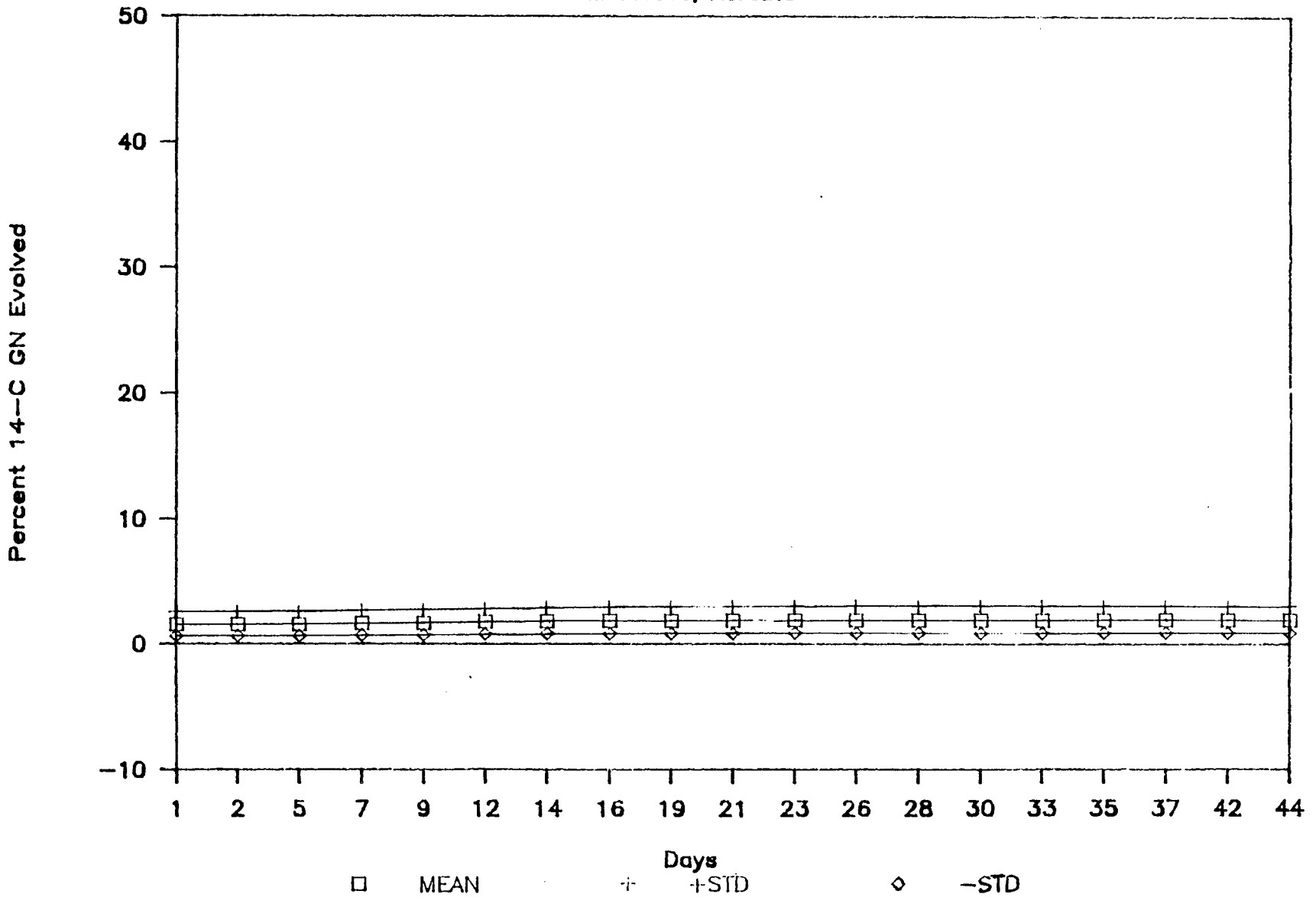
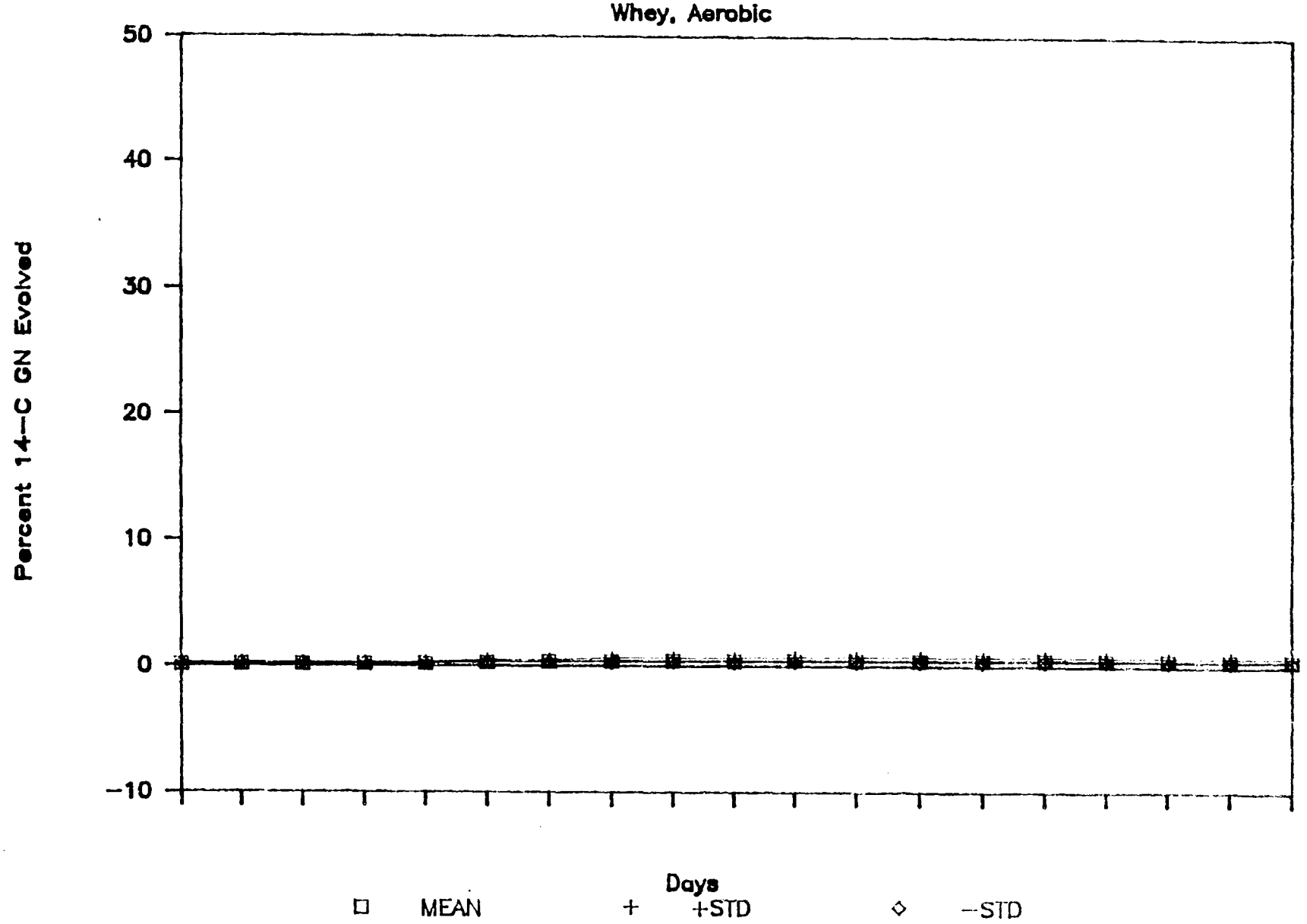

Volatilization of Guanidine Nitrate
Sterile, Aerobic
Percent 14-C GN Evolved
50
40
30
20
10
0
-10
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
-STD

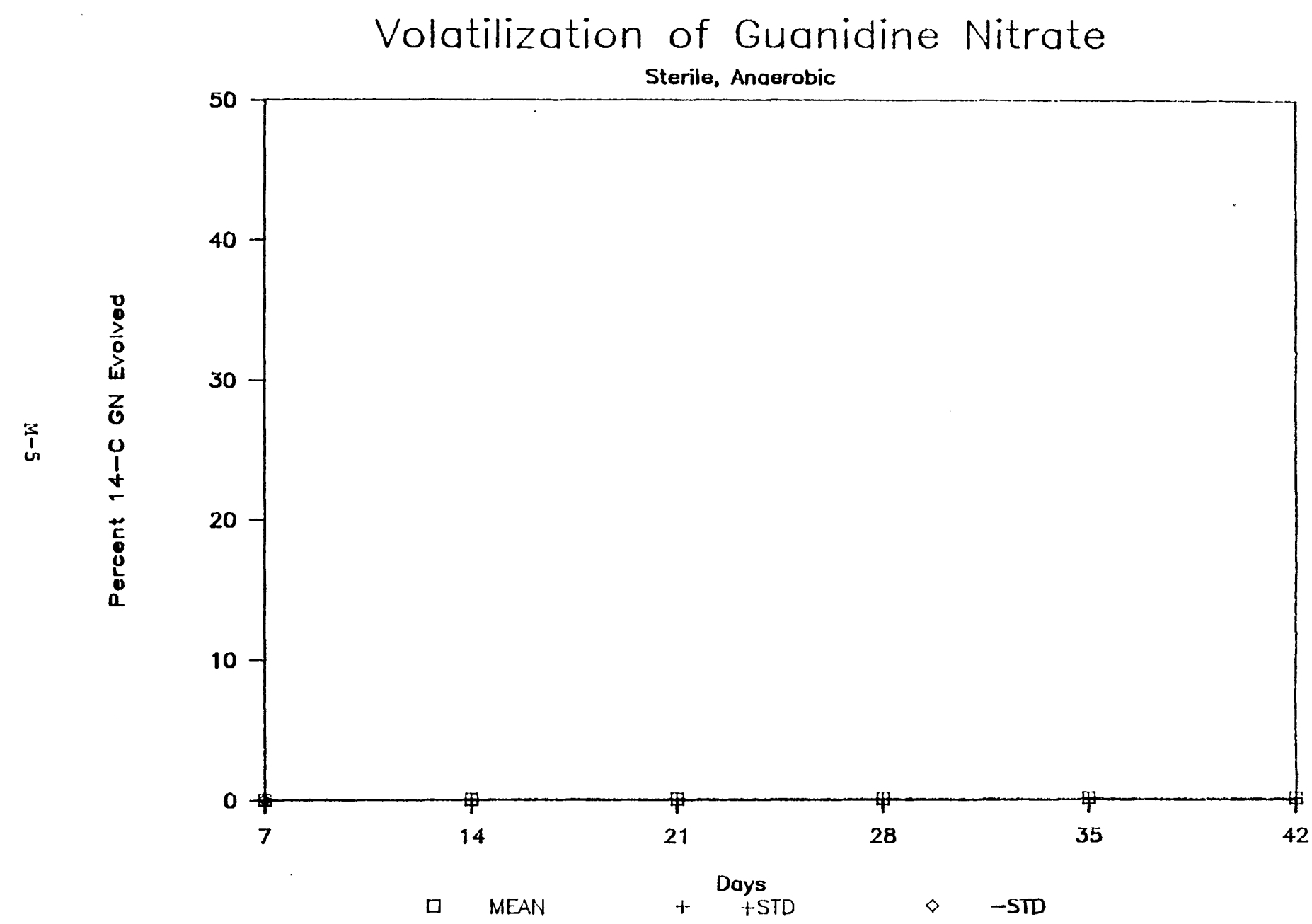
Figure 4-13


Volatilization of Guanidine Nitrate
Dextrose, Aerobic
Percent 14-C GN Evolved
50
40
30
20
10
0
-10
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
-STD

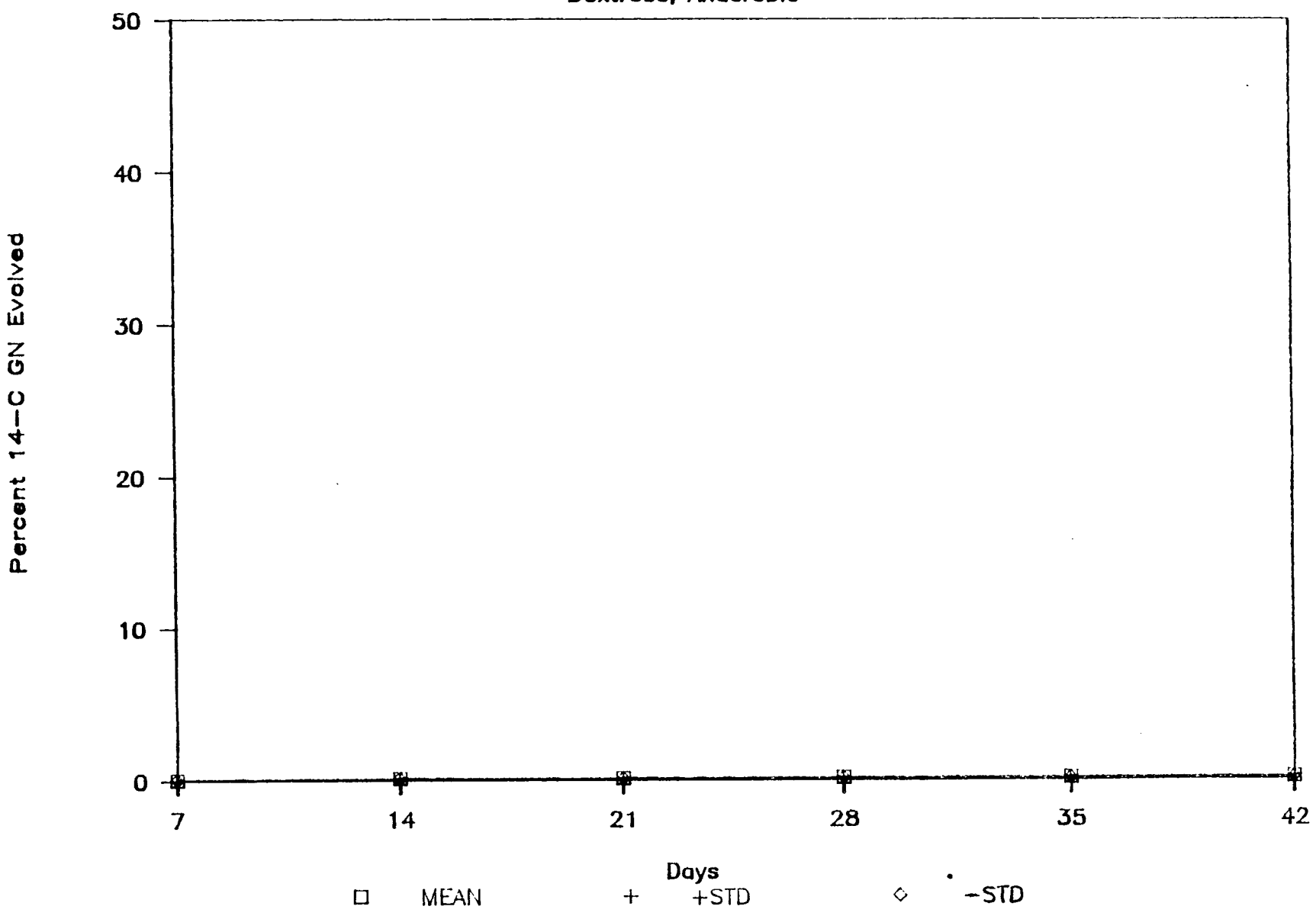

Volatilization of Guanidine Nitrate
Molasses, Aerobic
Percent 14—C GN Evolved
50
40
30
20
10
0
−10
1
2
5
7
9
12
14
16
19
21
23
26
28
30
33
35
37
42
44
Days
MEAN
+STD
−STD

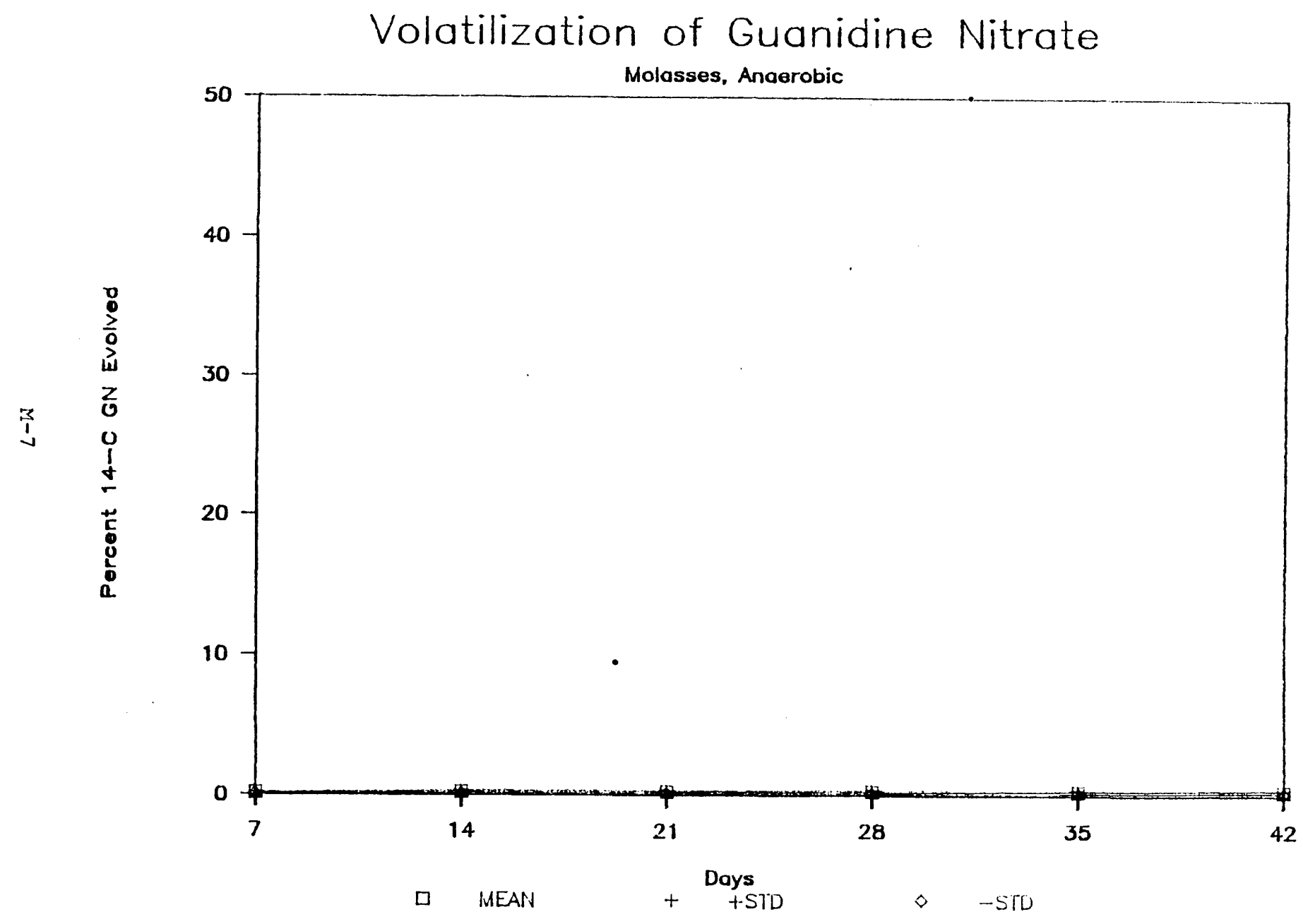
# Volatilization of Guanidine Nitrate



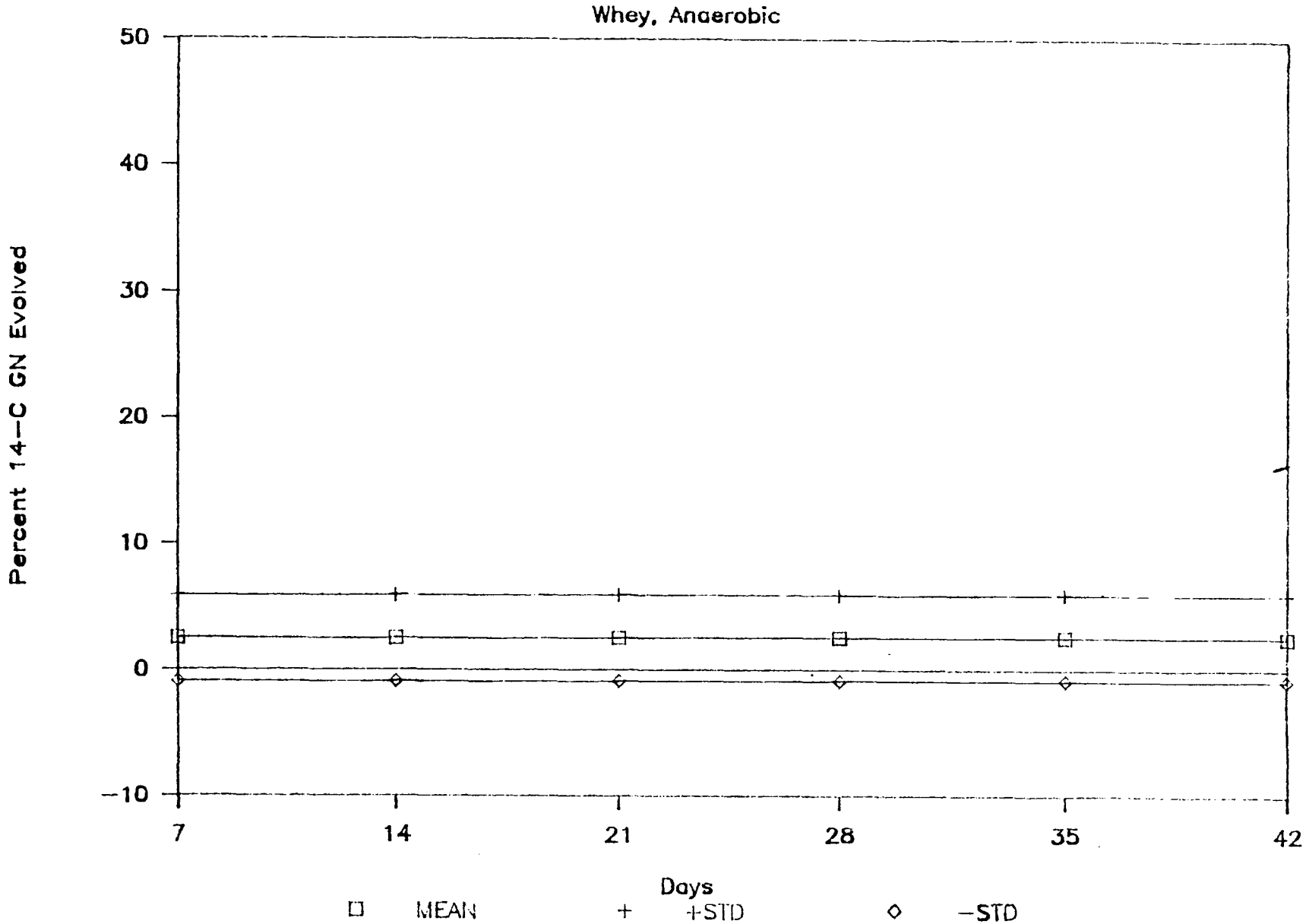

Volatilization of Guanidine Nitrate
Sterile, Anaerobic
Percent 14-C GN Evolved
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
-STD

# Volatilization of Guanidine Nitrate



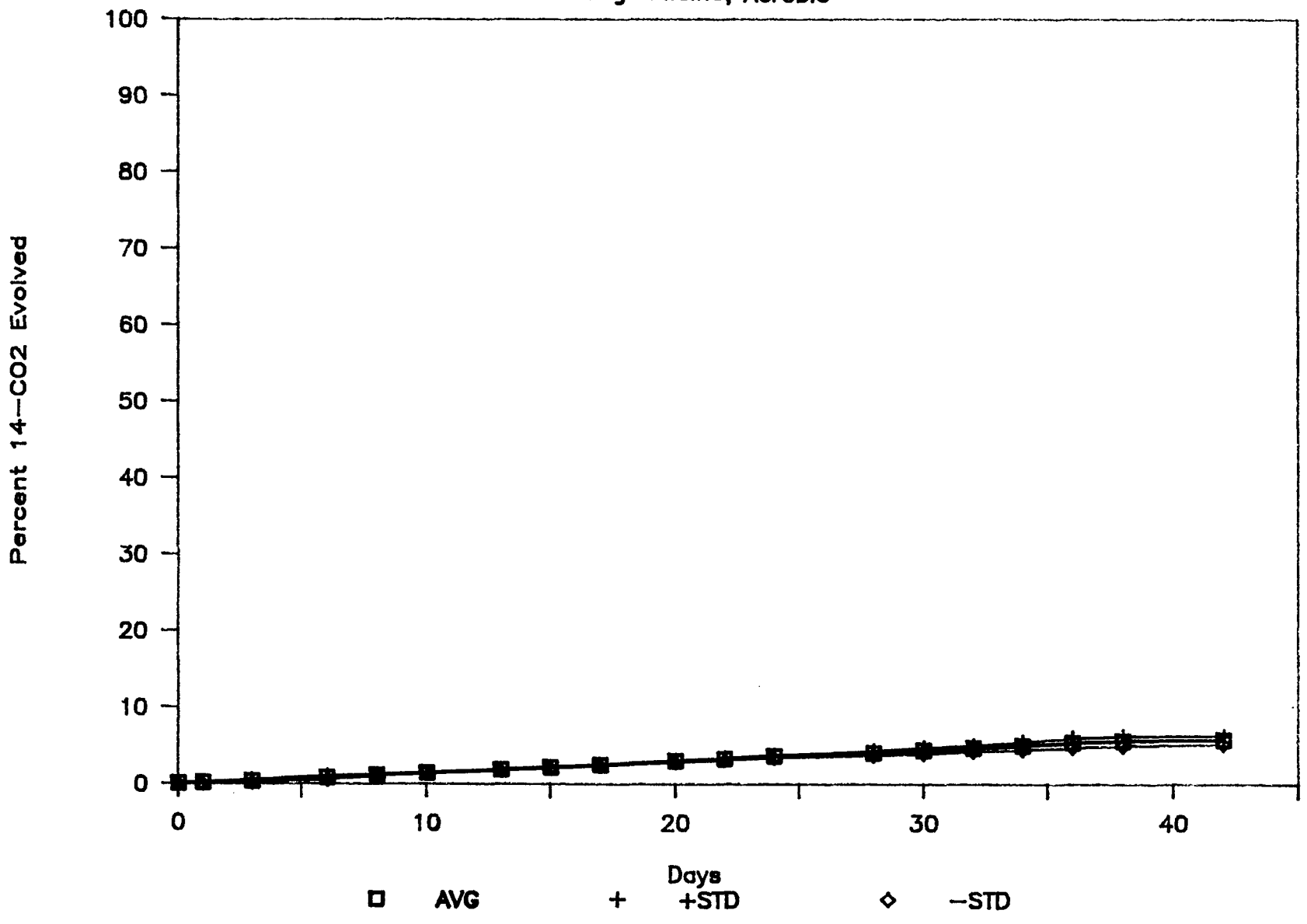

Volatilization of Guanidine Nitrate
Molasses, Anaerobic
Percent 14-C GN Evolved
50
40
30
20
10
0
7
14
21
28
35
42
Days
MEAN
+STD
-STD

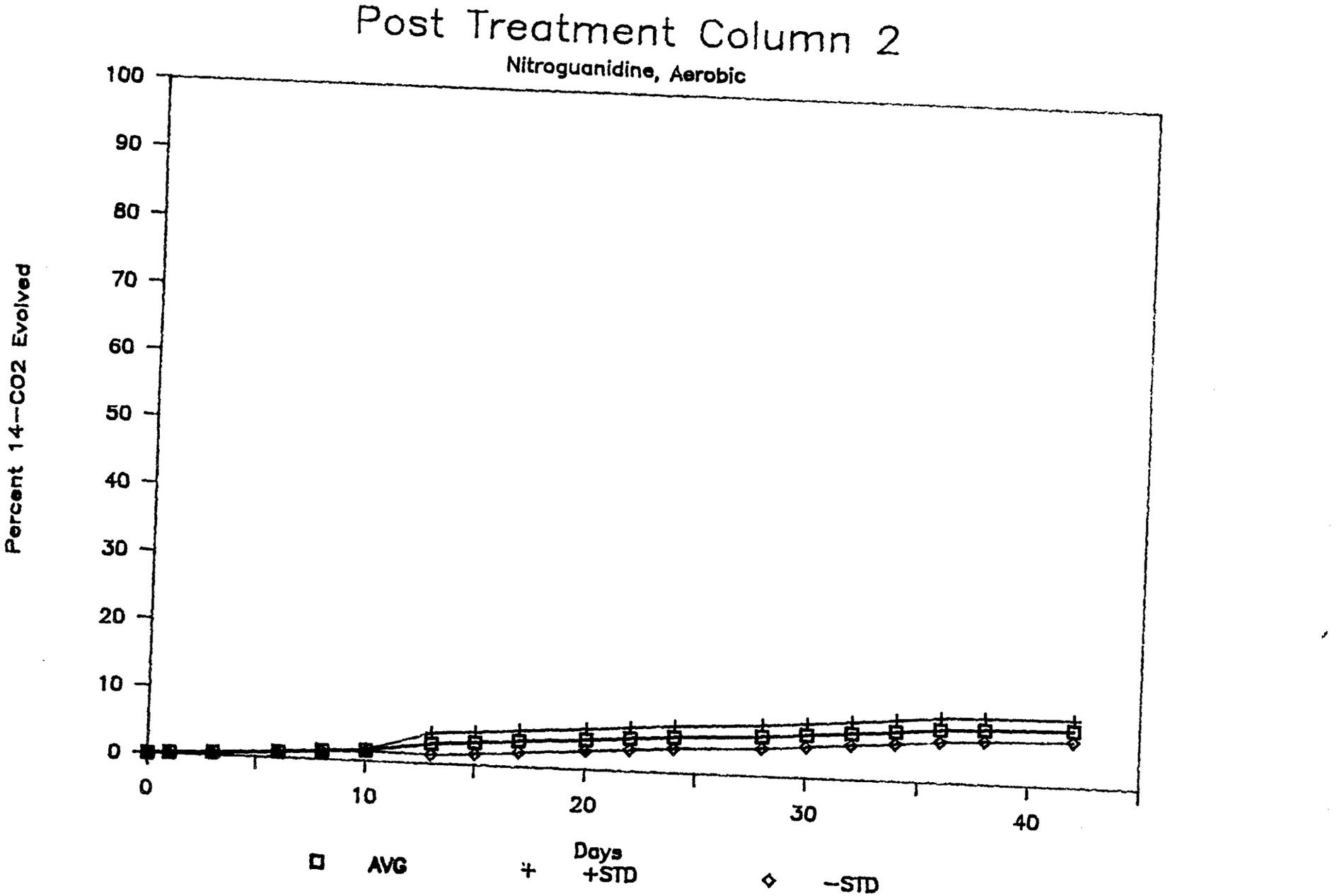
# Volatilization of Guanidine Nitrate

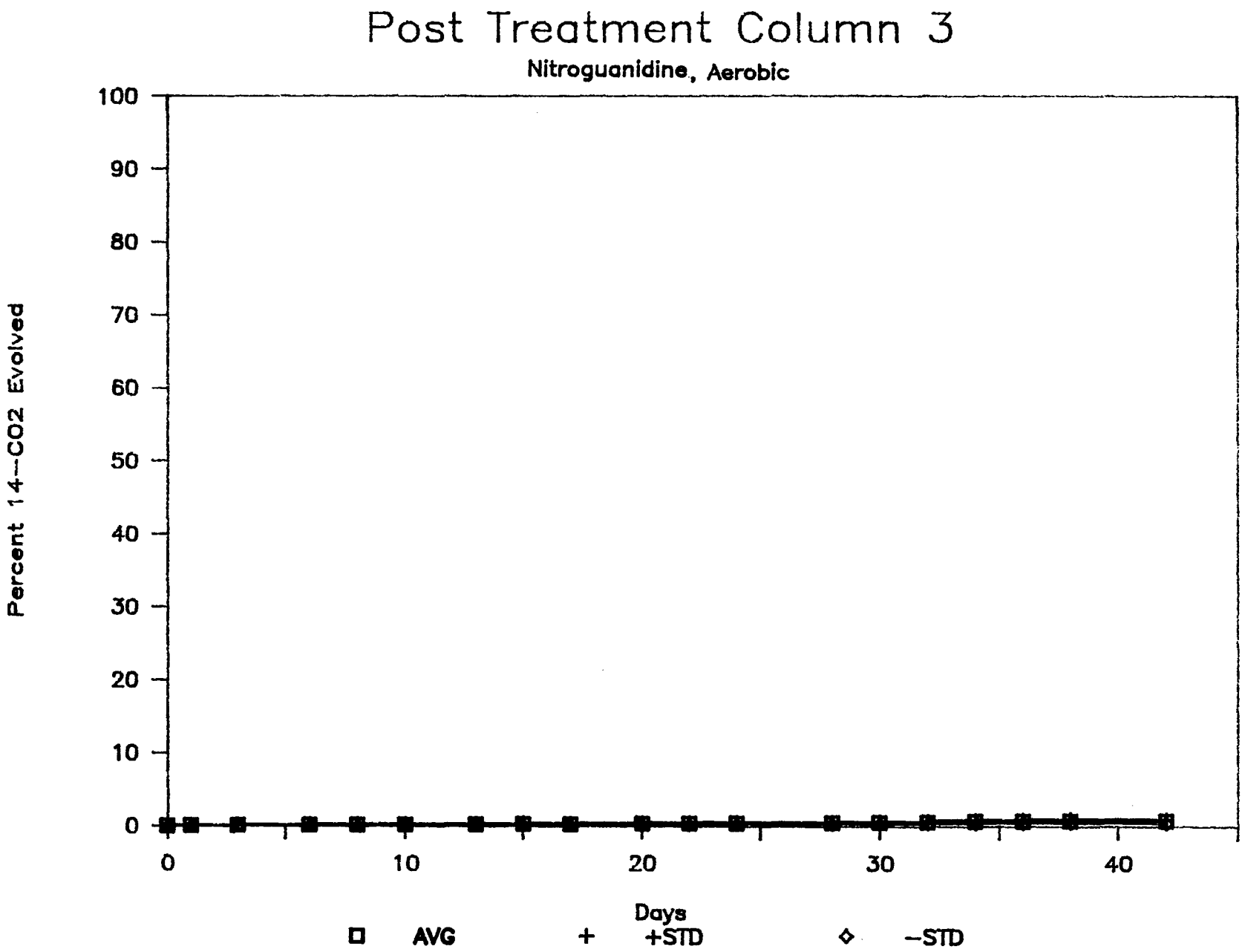


# APPENDIX N

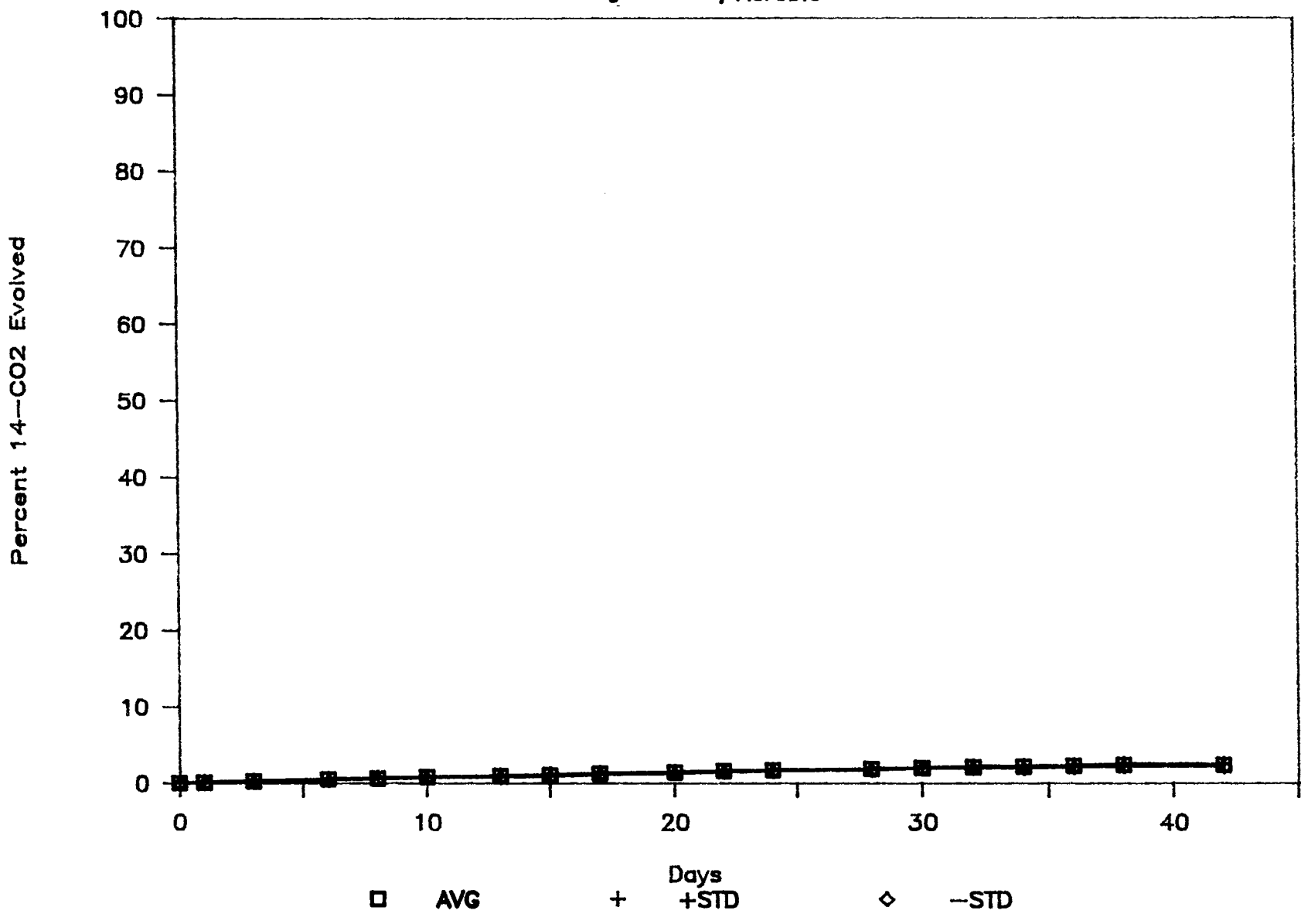
## GRAPHS OF NITROGUANIDINE MINERALIZATION IN POST-TREATMENT SOIL - AEROBIC AND ANAEROBIC CONDITIONS

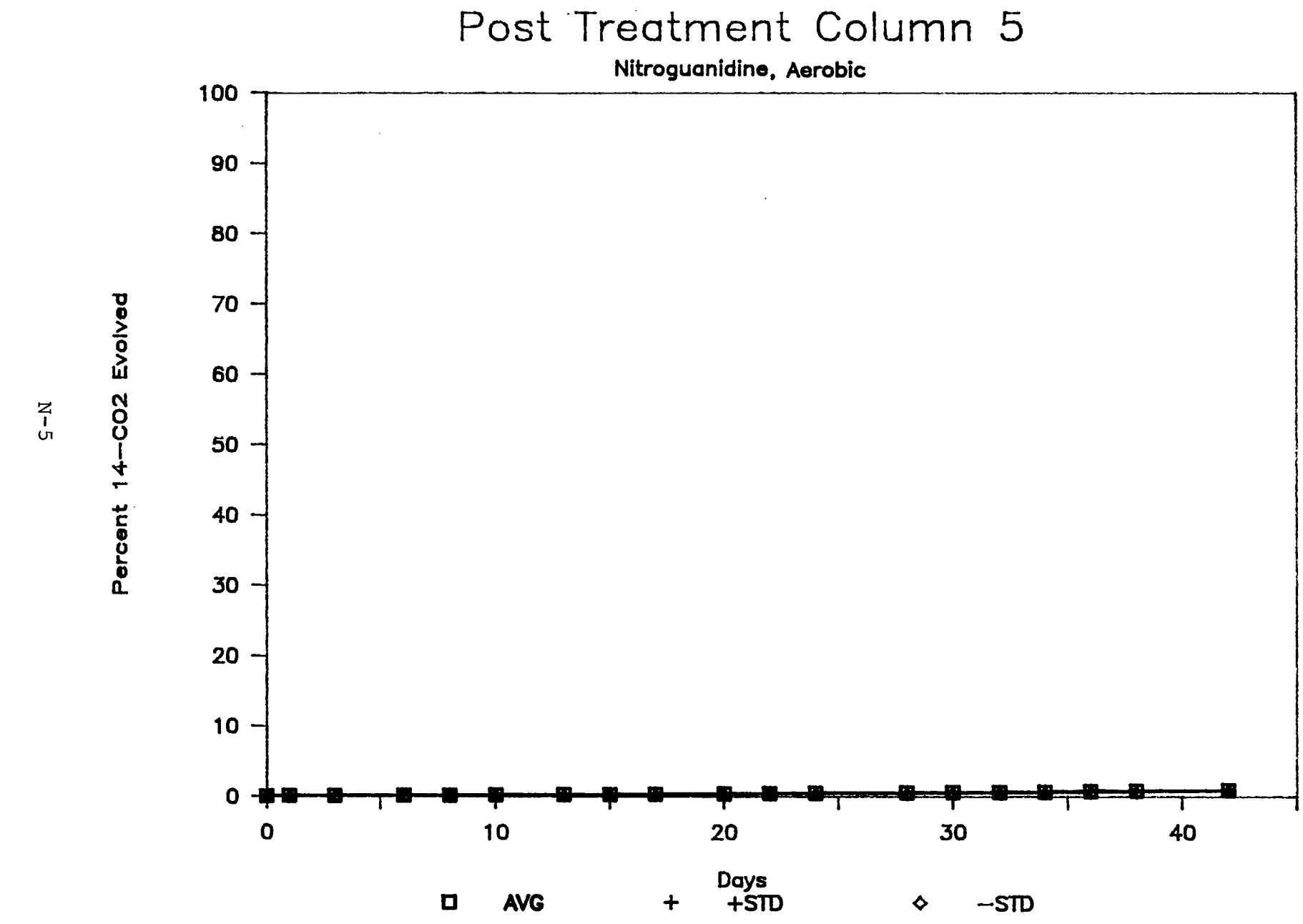

Post Treatment Column 1
Nitroguanidine, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD


Post Treatment Column 2
Nitroguanidine, Aerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
-STD

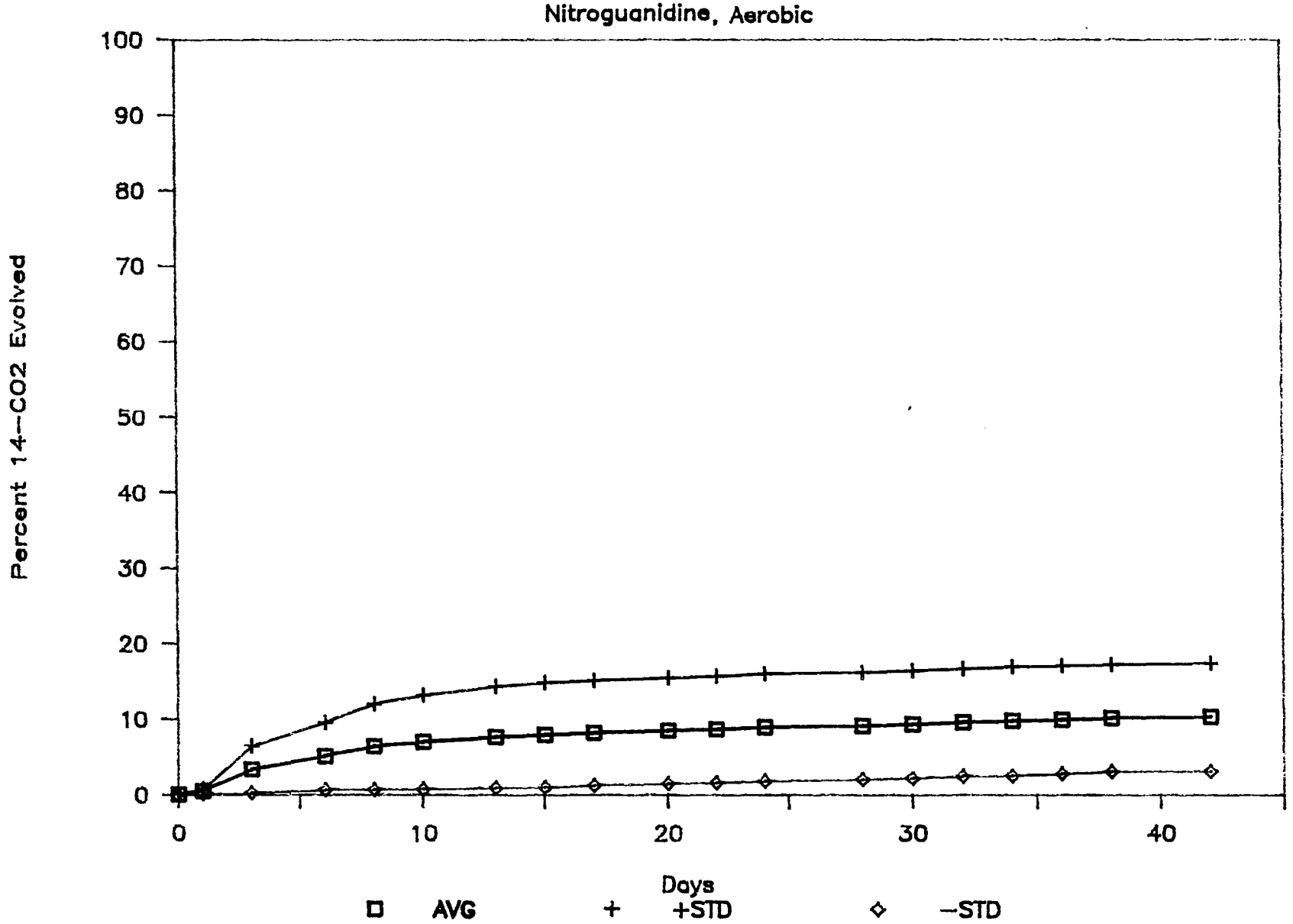

Post Treatment Column 3
Nitroguanidine, Aerobic
Percent 14-CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
-STD

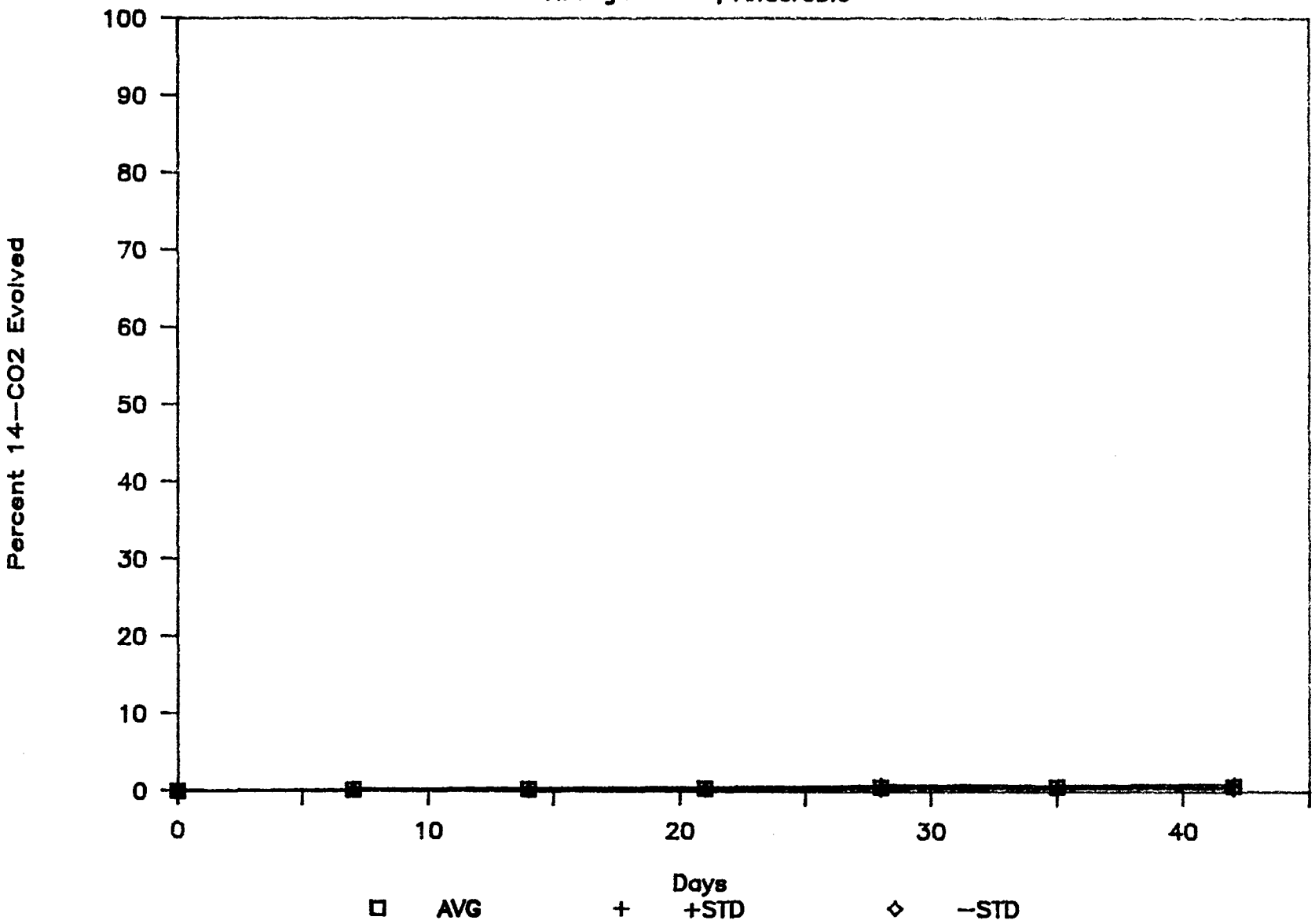
# Post Treatment Column 4



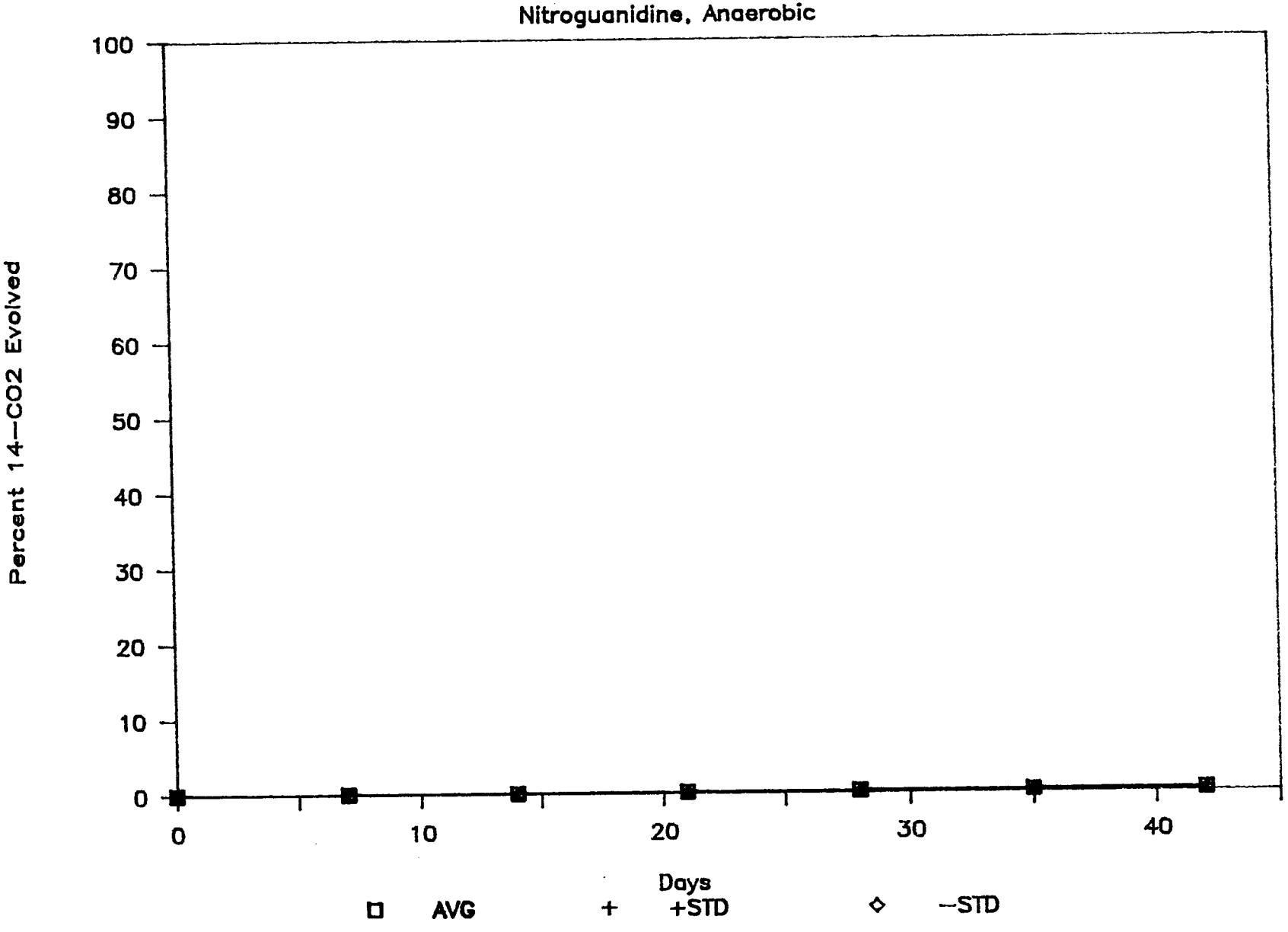

Post Treatment Column 5
Nitroguanidine, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD

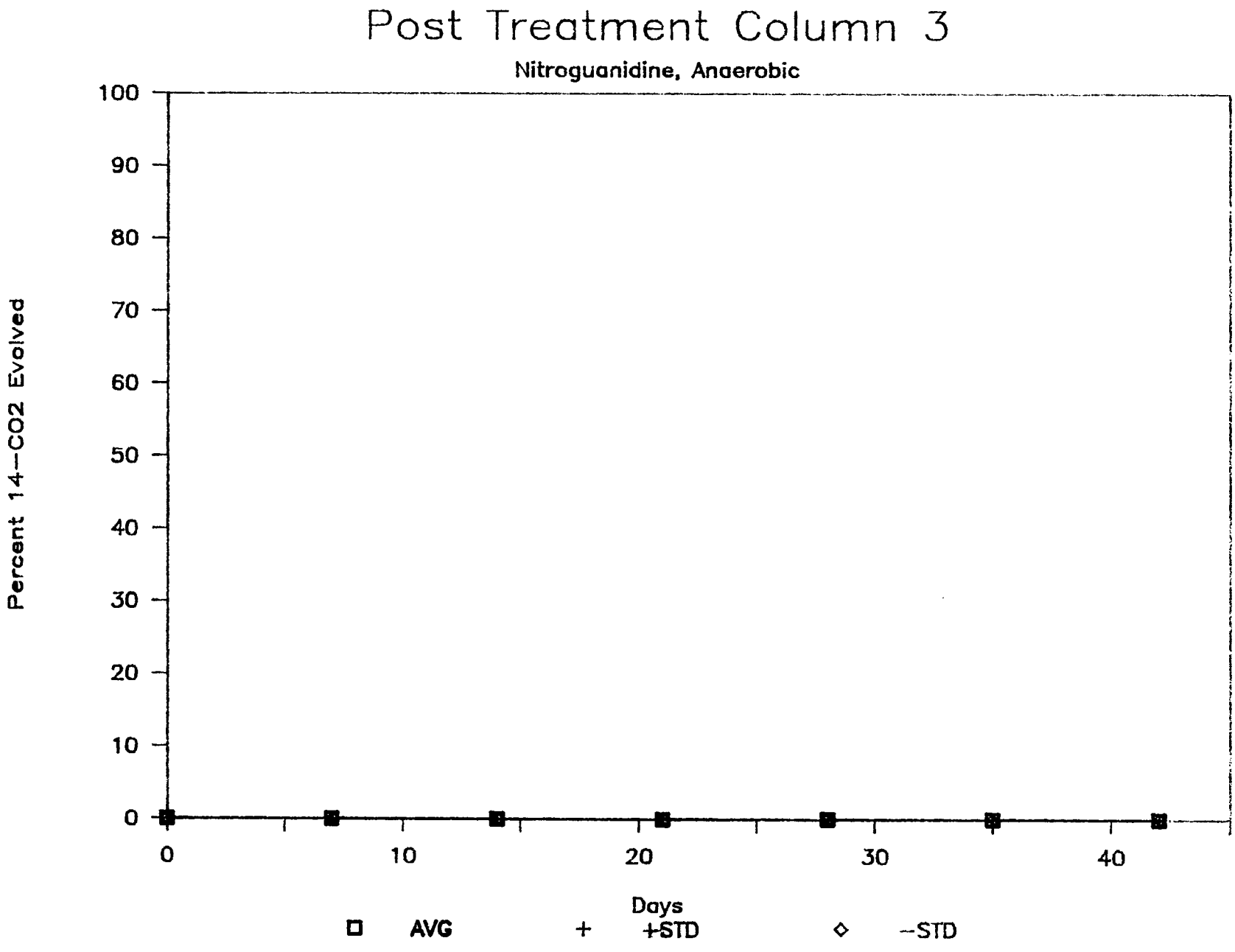
# Post Treatment Column 6



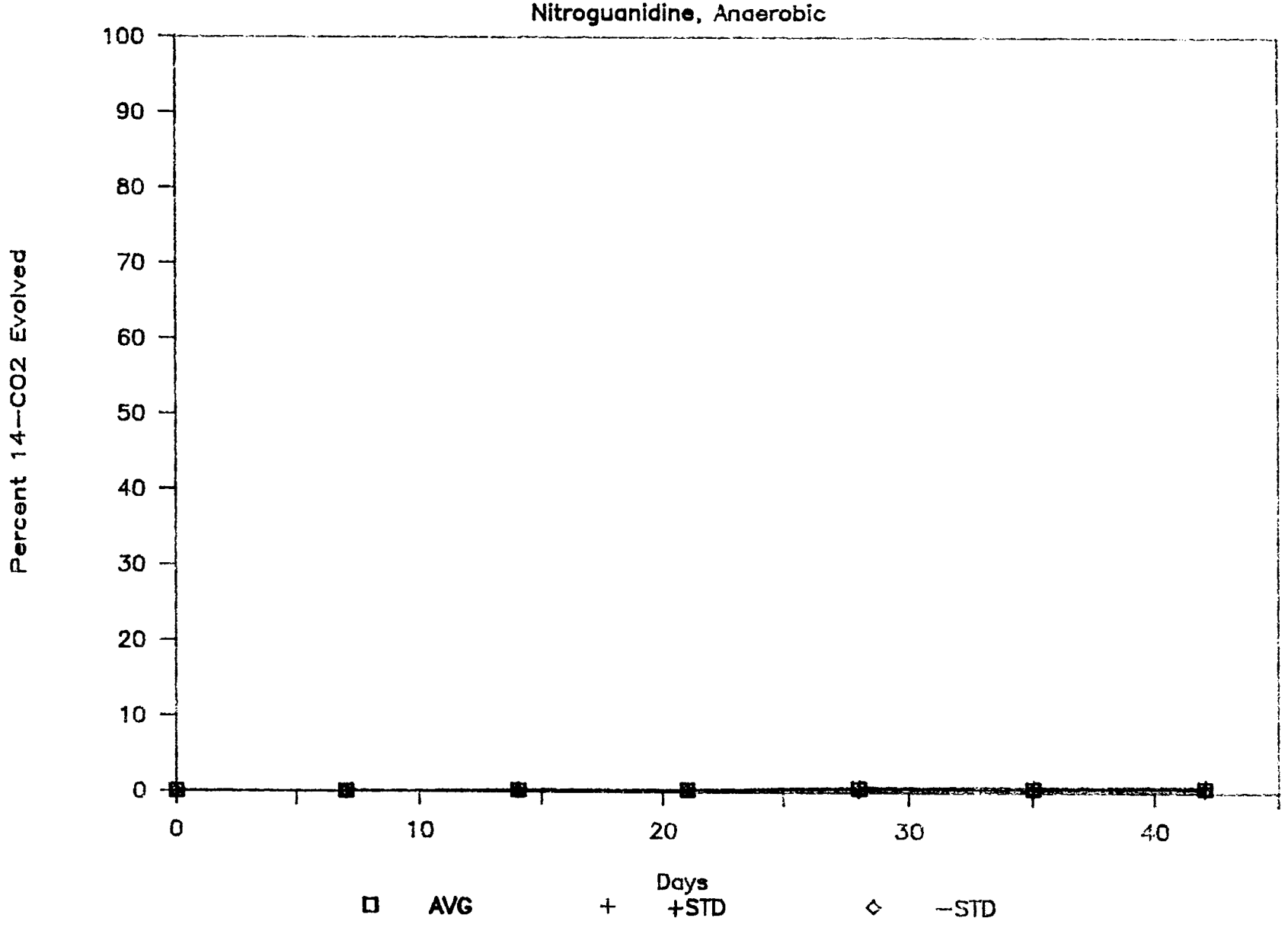

Post Treatment Column 1
Nitroguanidine, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD

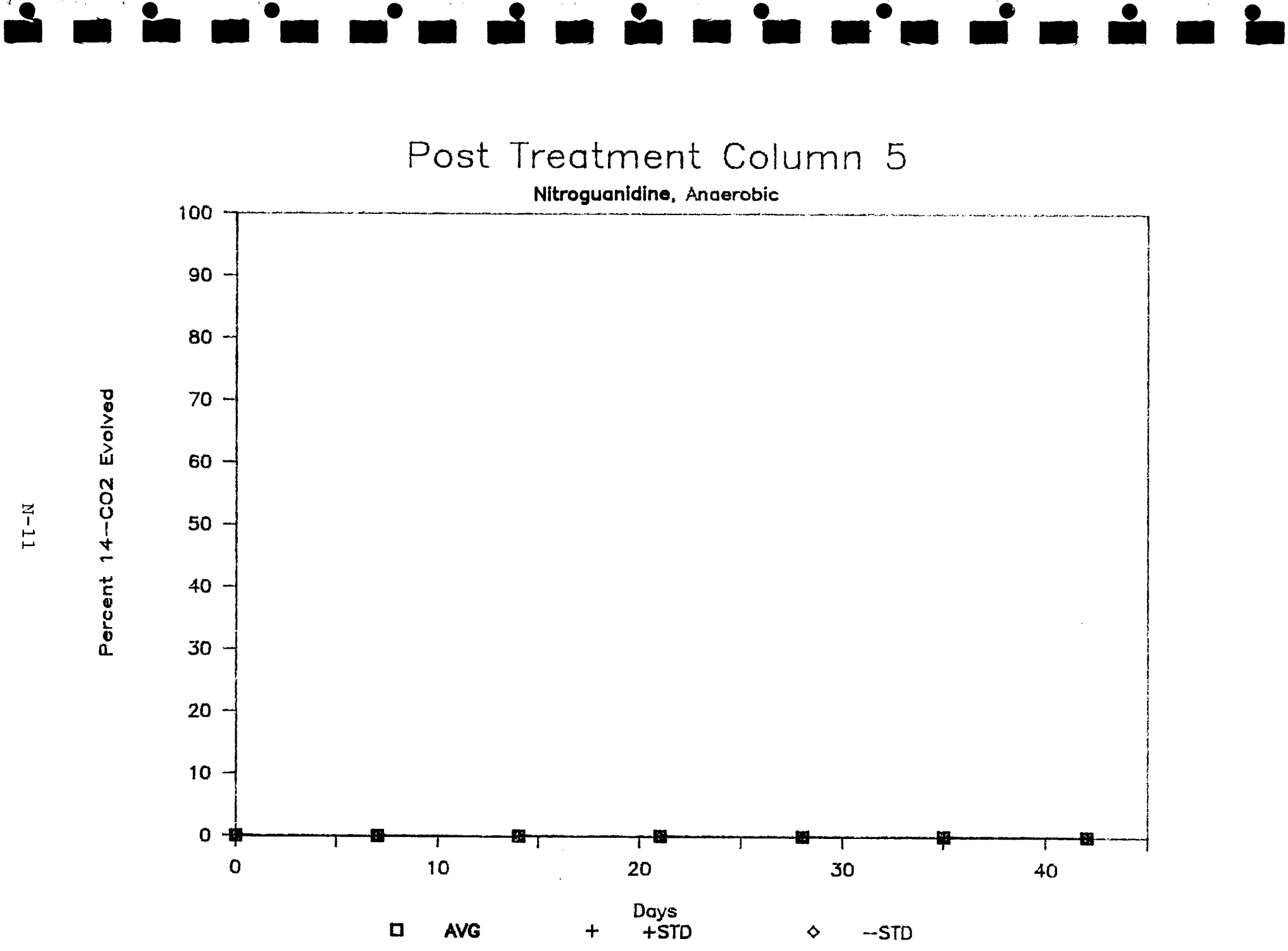
# Post Treatment Column 2



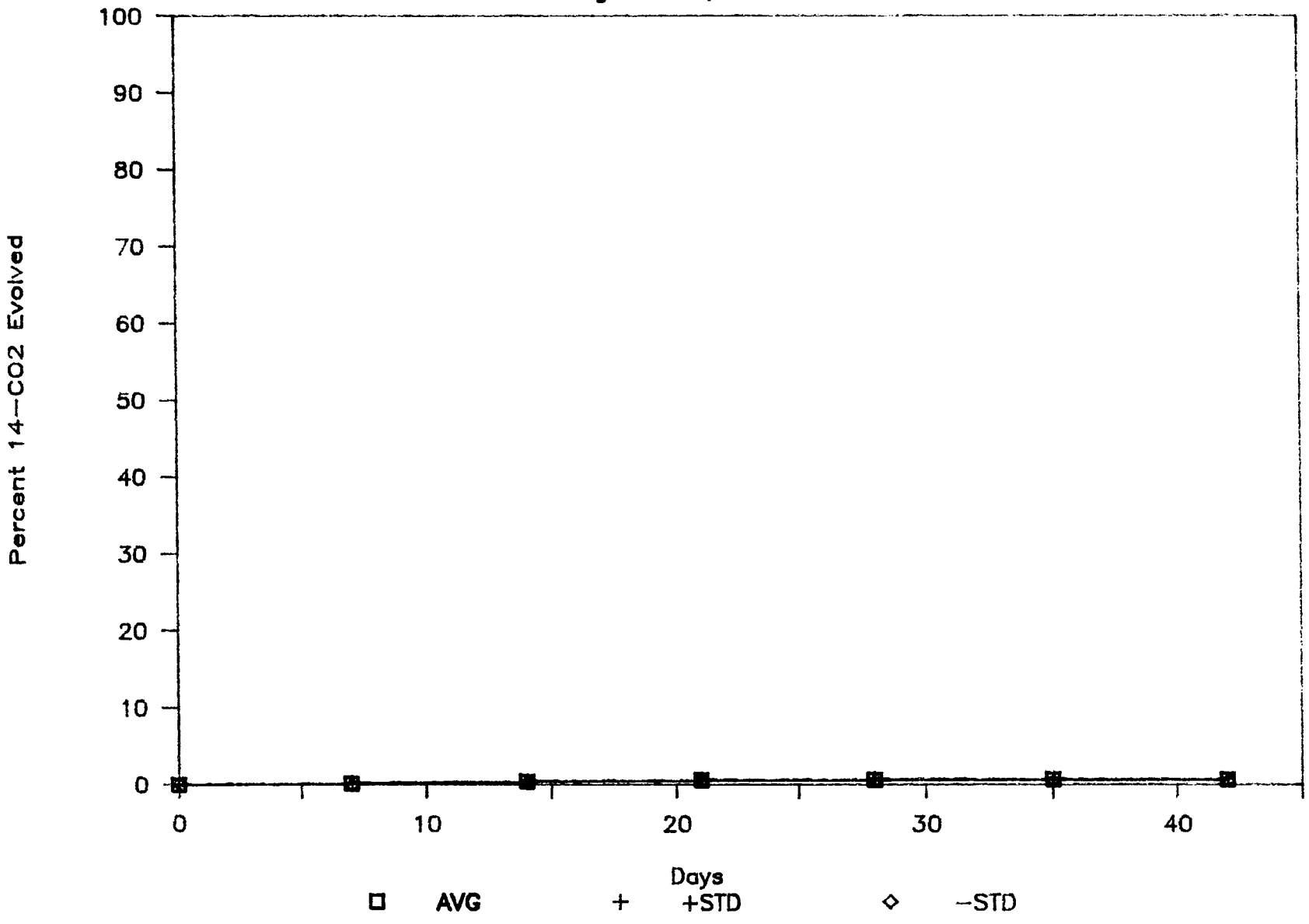

Post Treatment Column 3
Nitroguanidine, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD

# Post Treatment Column 4



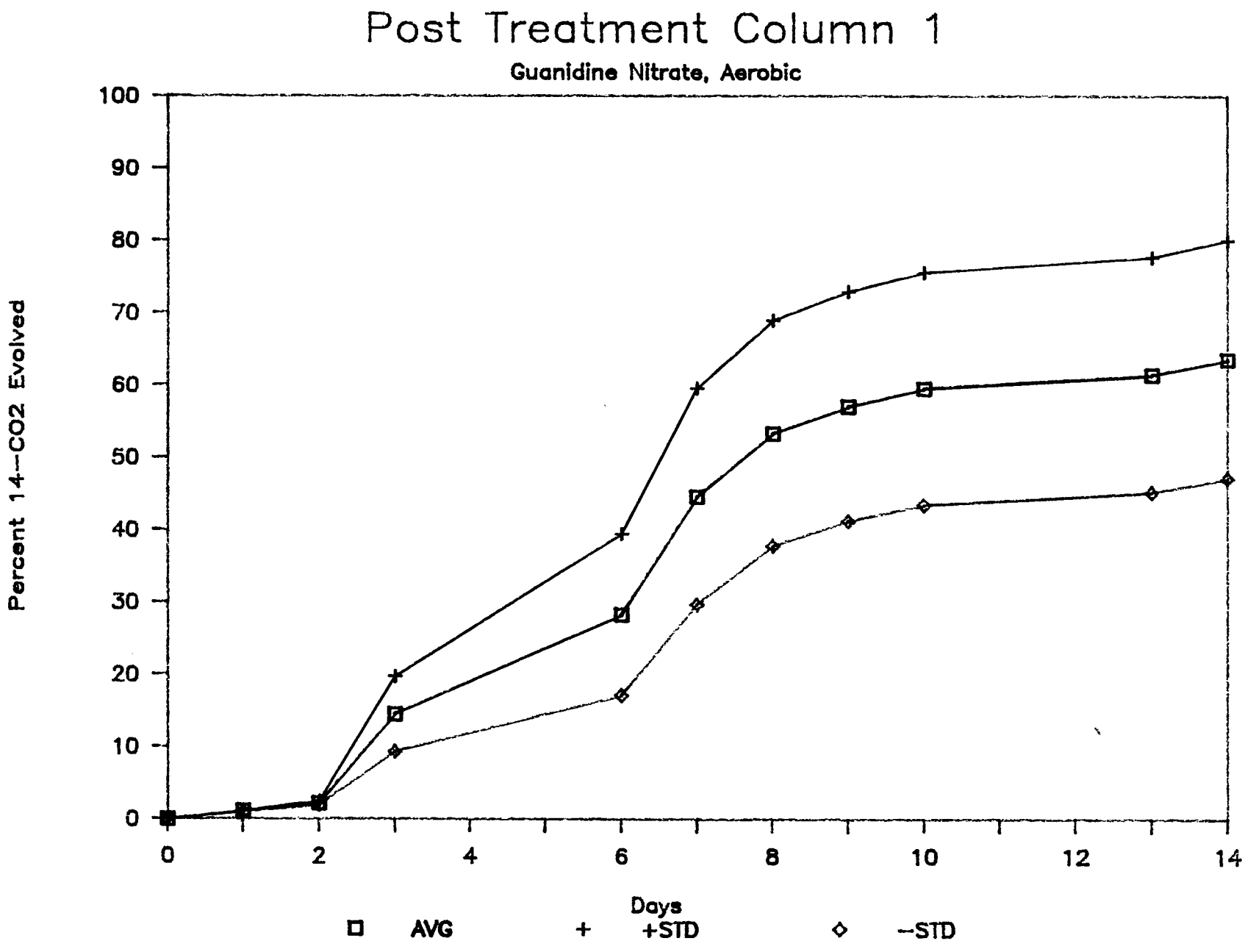

Post Treatment Column 5
Nitroguanidine, Anaerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
--STD

# Post Treatment Column 6

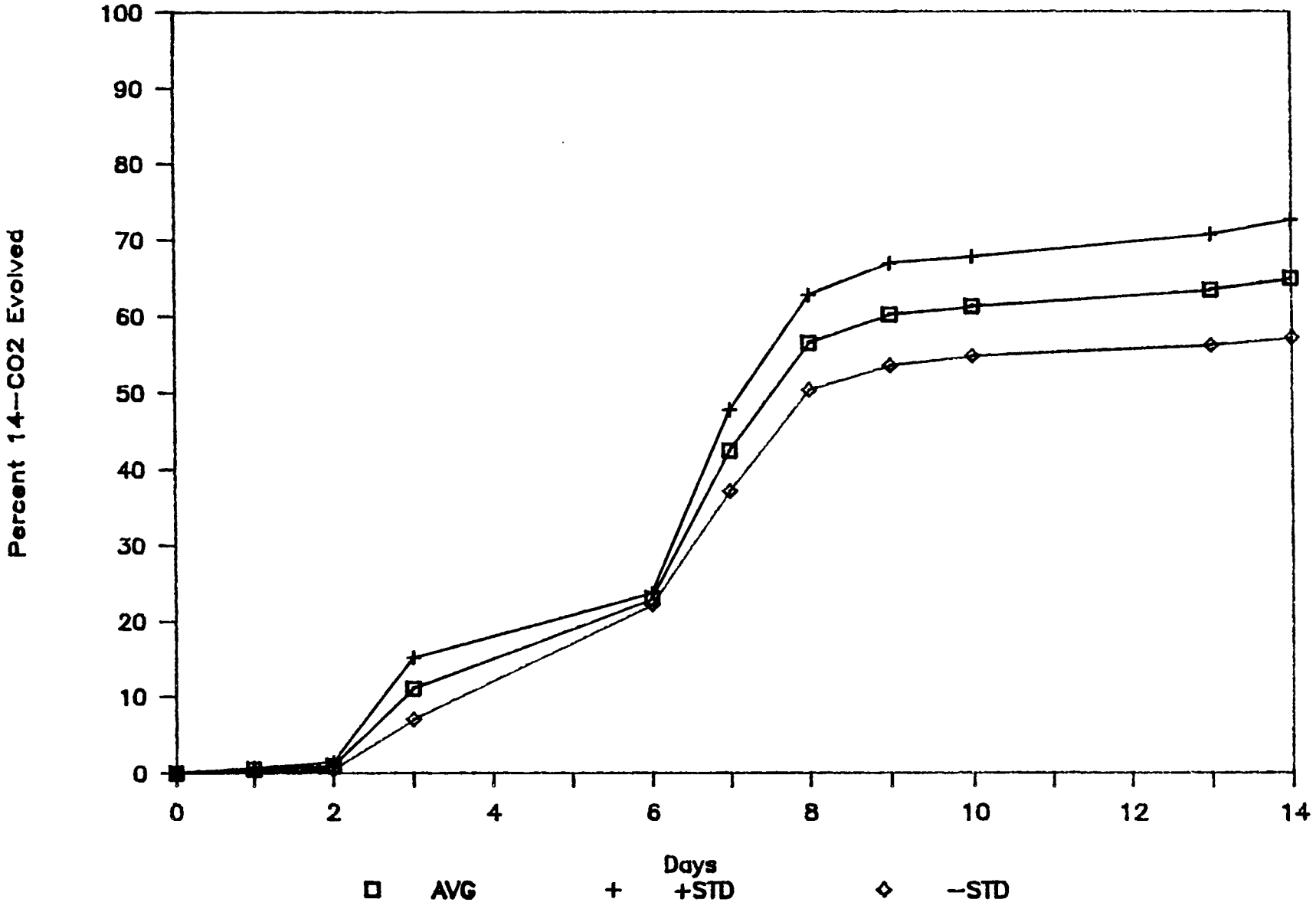


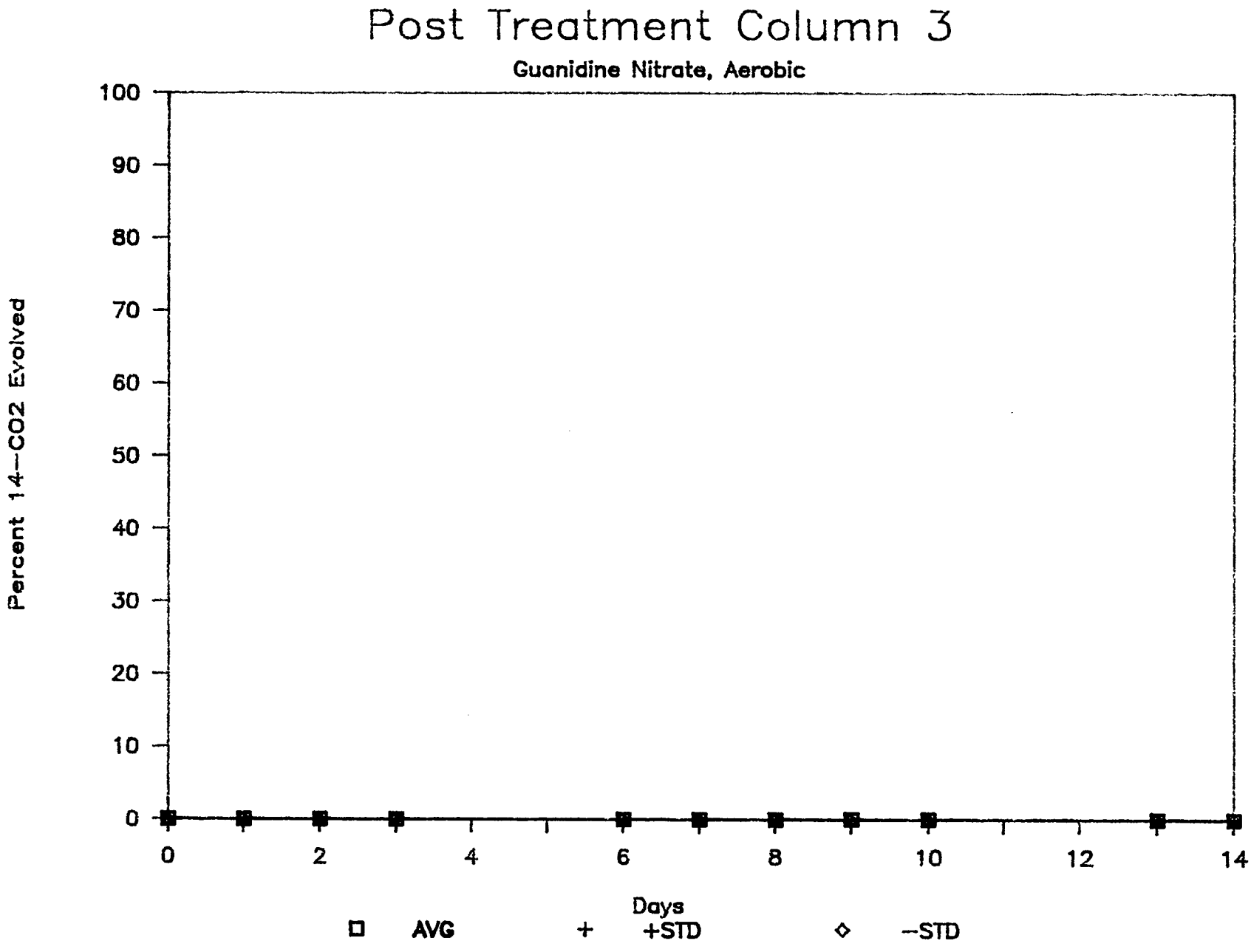
# APPENDIX O

## GRAPHS OF GN MINERALIZATION IN POST-TREATMENT SOIL

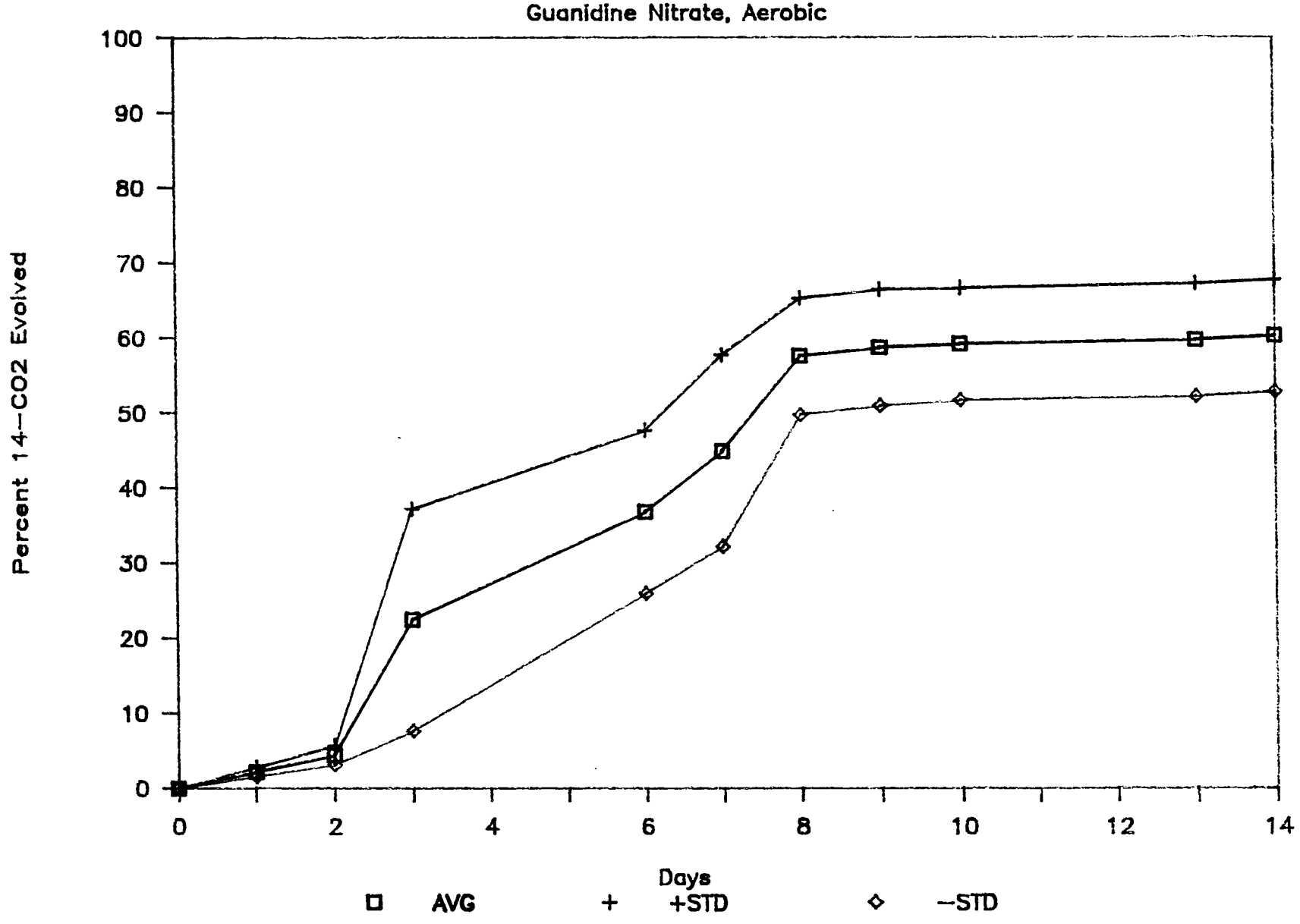

Post Treatment Column 1
Guanidine Nitrate, Aerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days
AVG
+STD
--STD

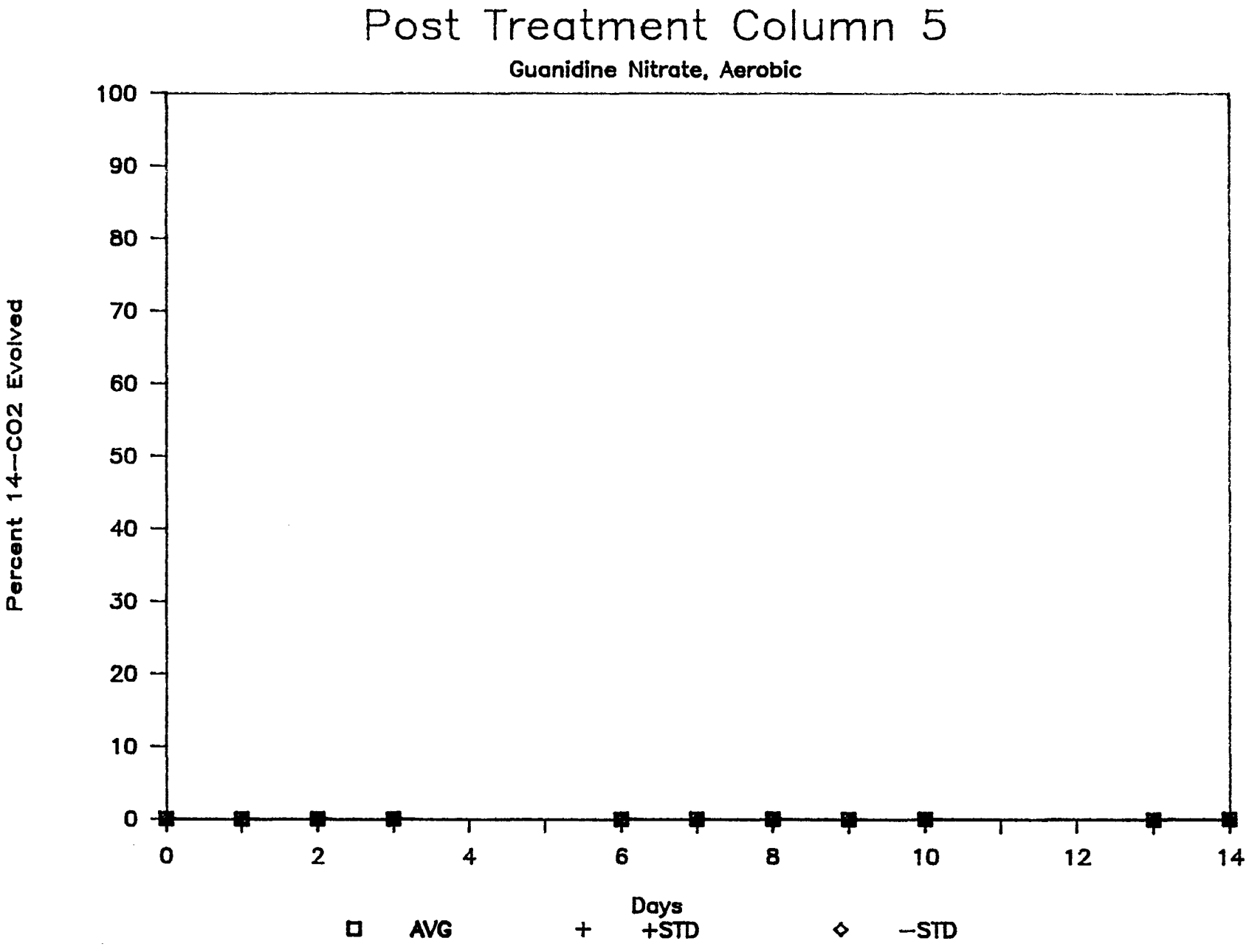
# Post Treatment Column 2



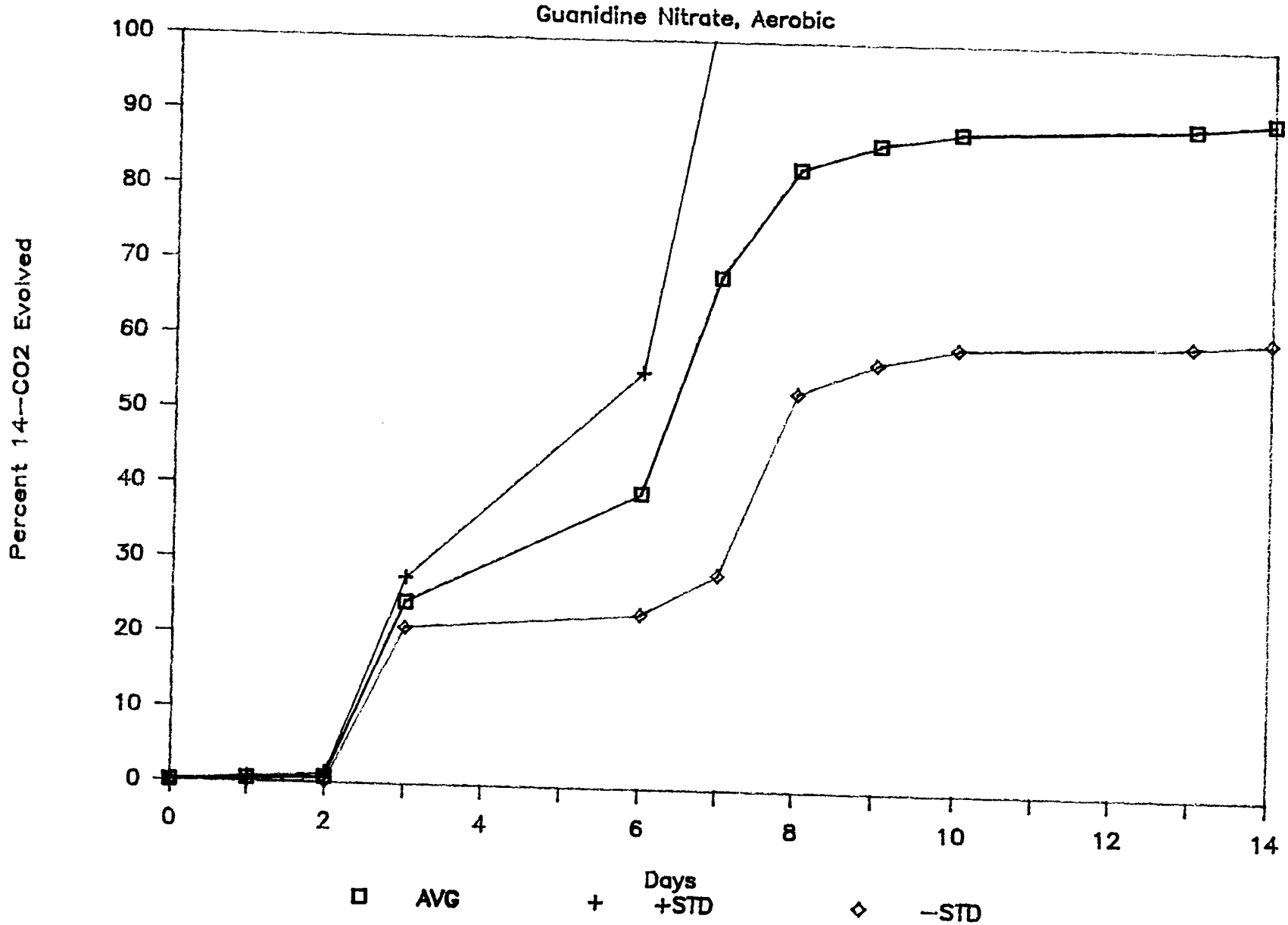

Post Treatment Column 3
Guanidine Nitrate, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days
AVG
+STD
—STD

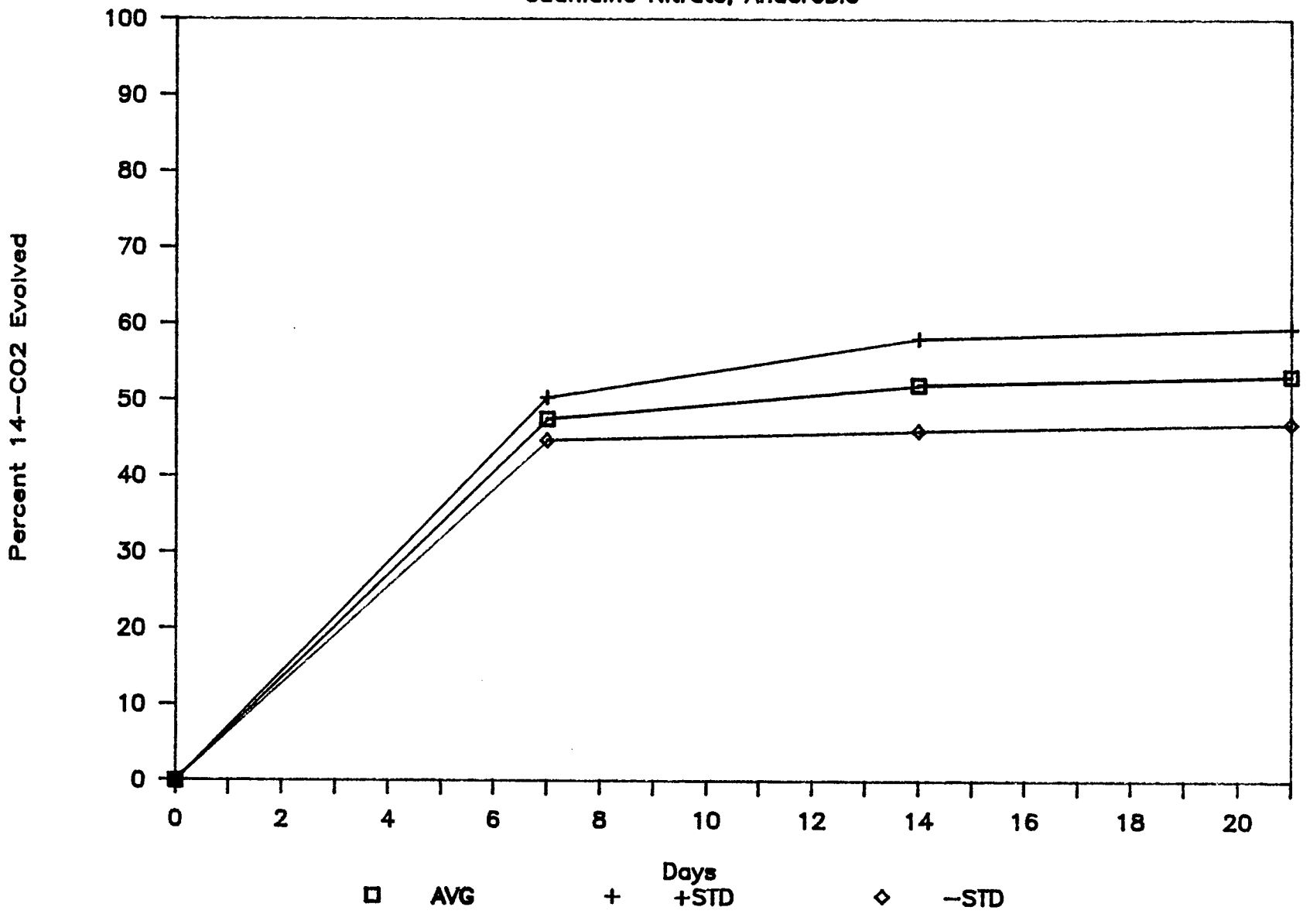
# Post Treatment Column 4

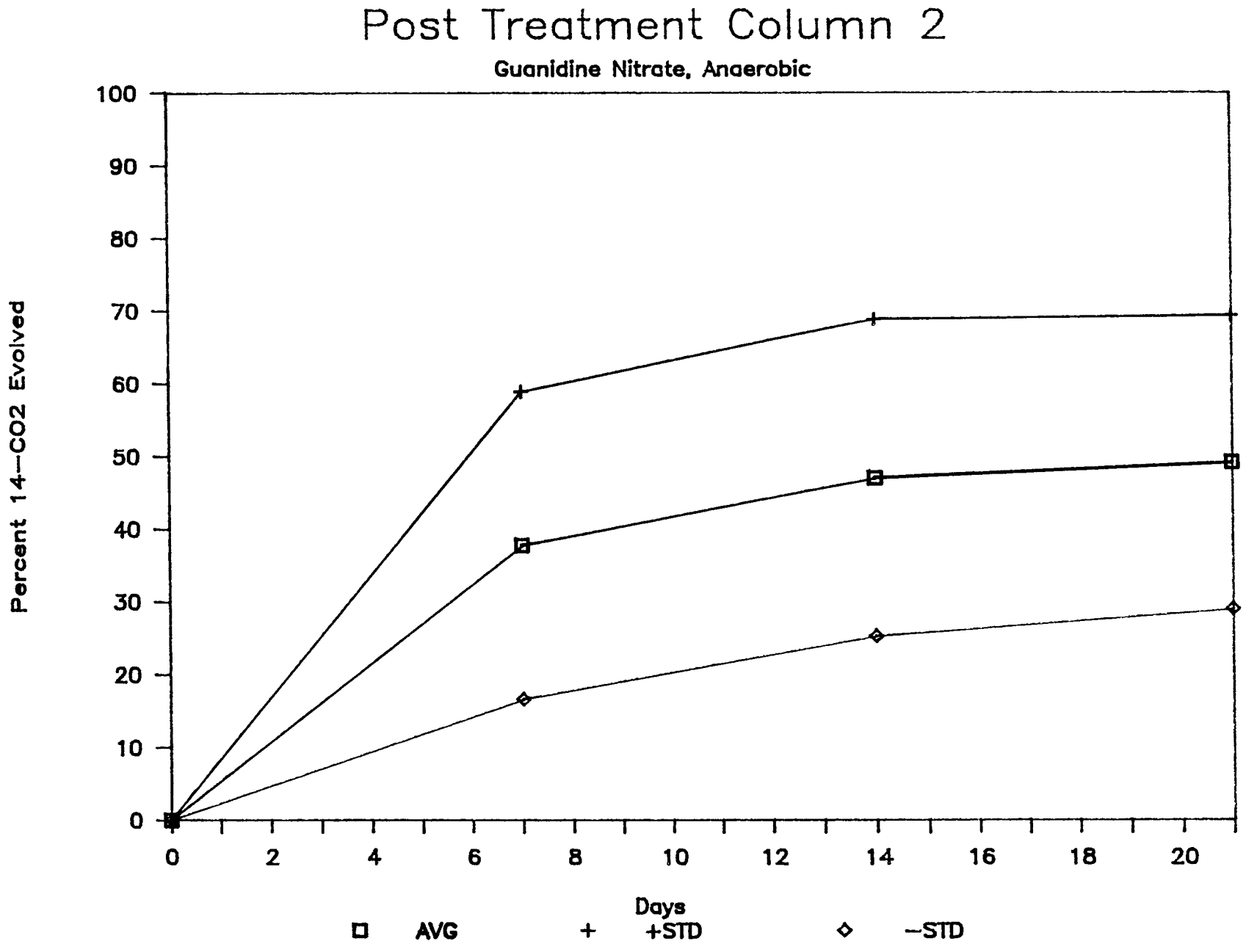



Post Treatment Column 5
Guanidine Nitrate, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Days
AVG
+STD
—STD

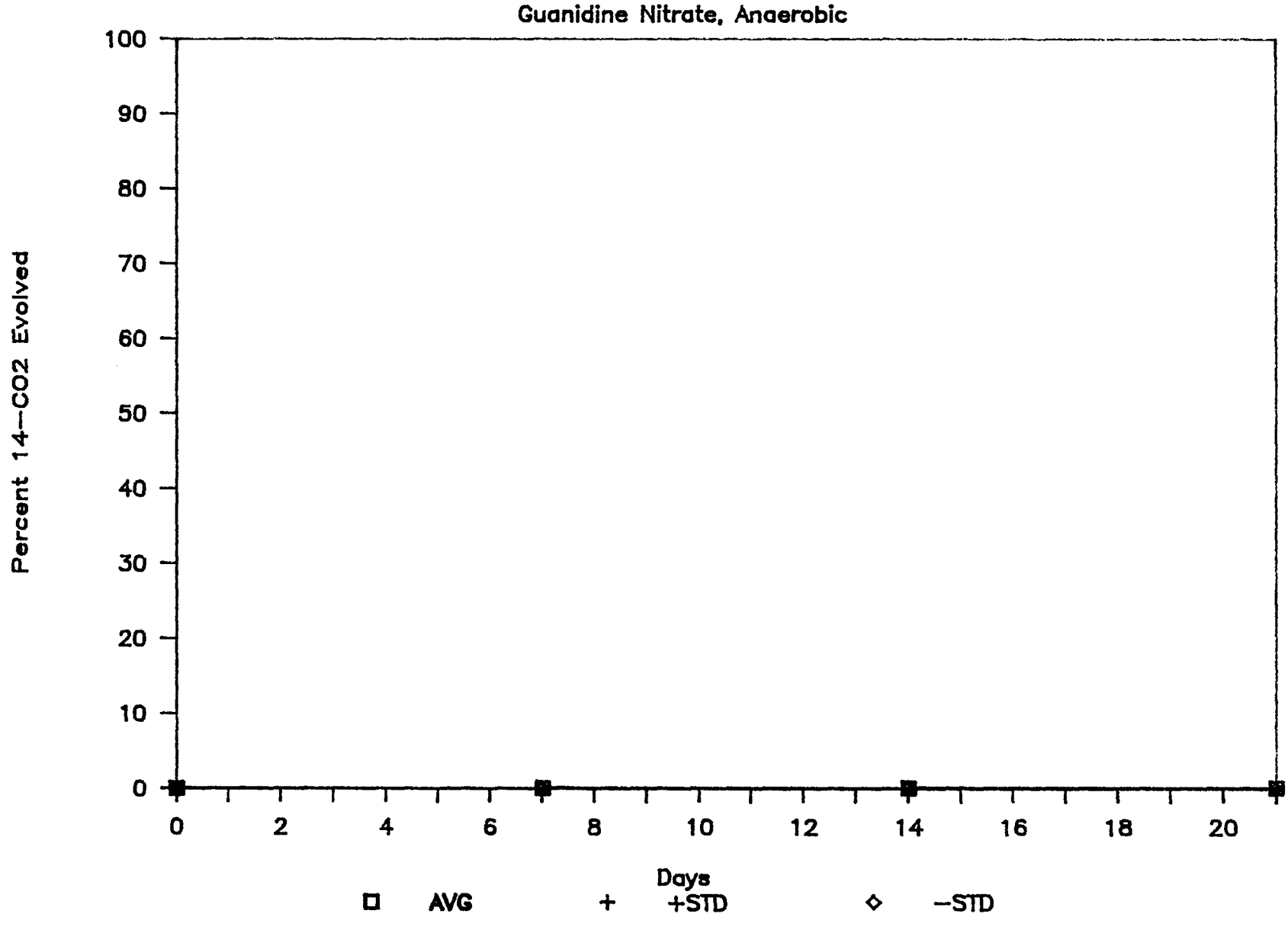
# Post Treatment Column 6

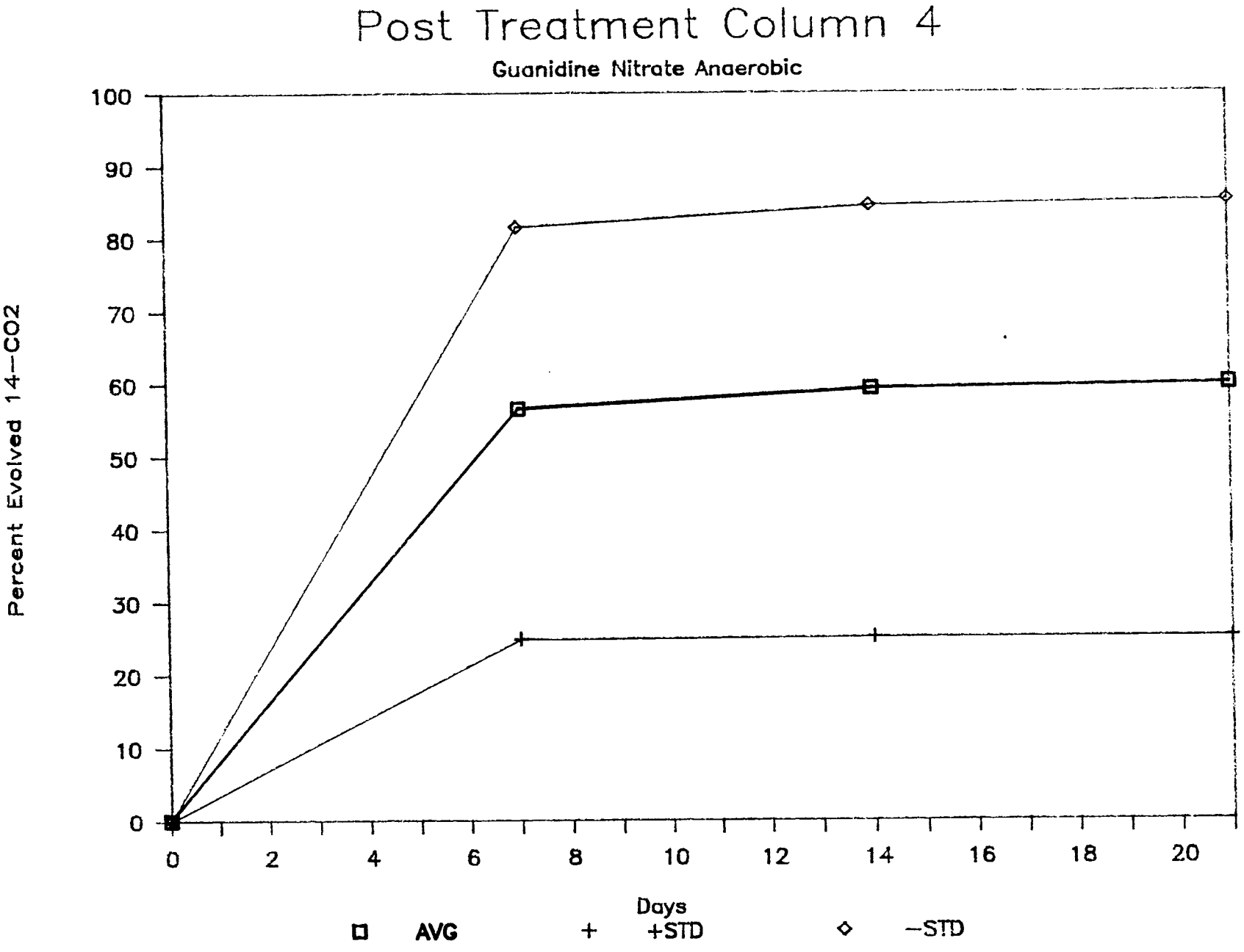


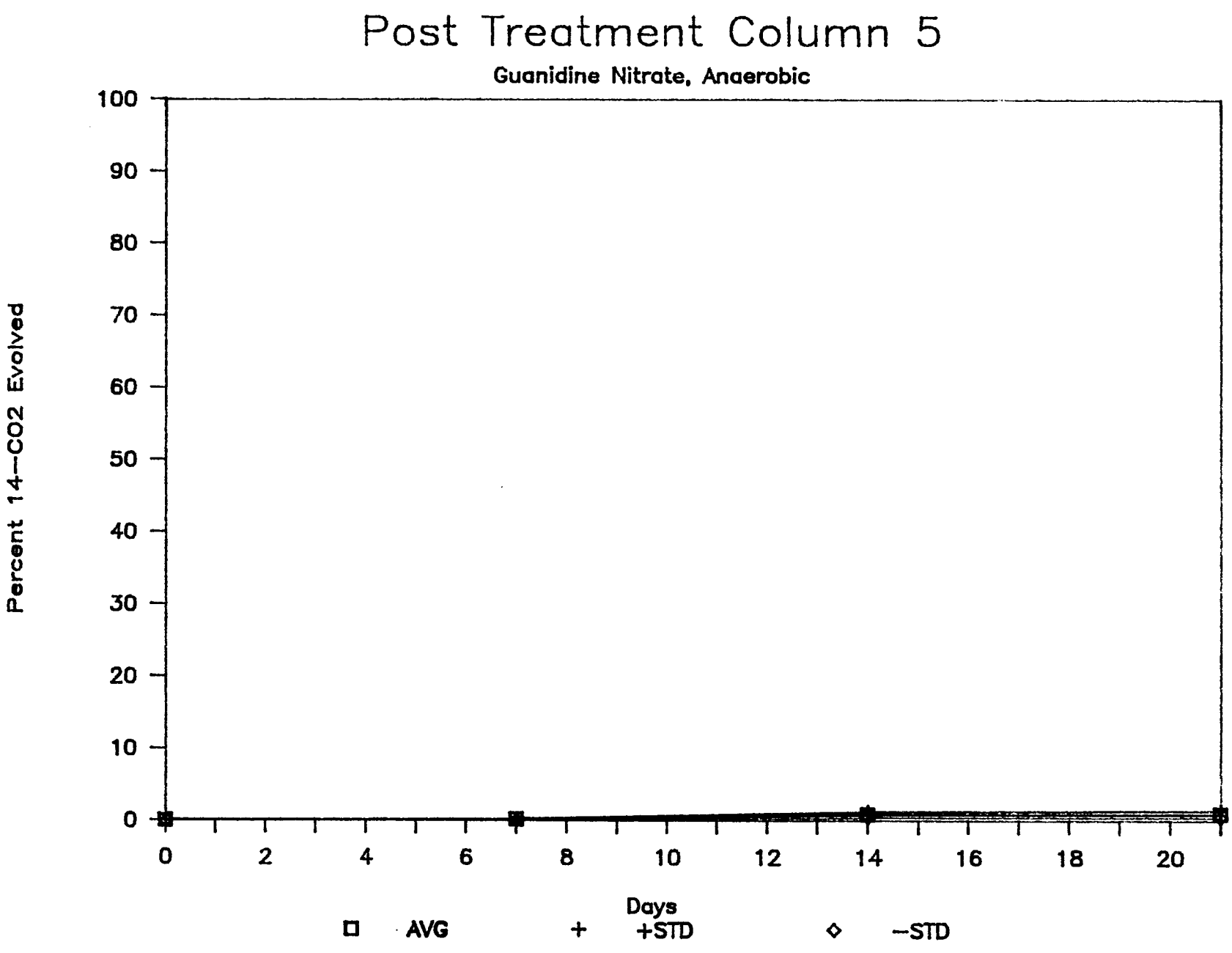

Post Treatment Column 1
Guanidine Nitrate, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

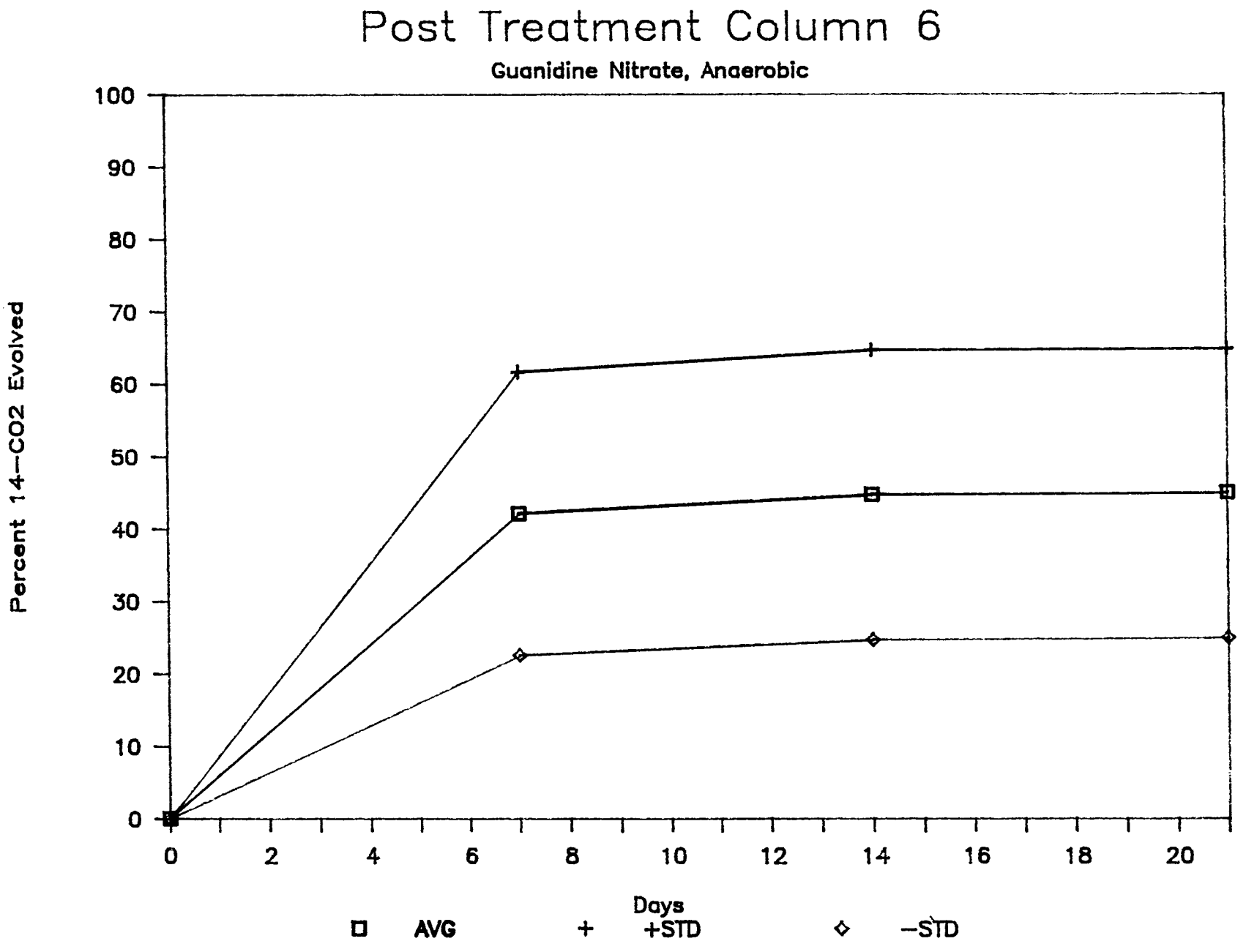

Post Treatment Column 2
Guanidine Nitrate, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

# Post Treatment Column 3



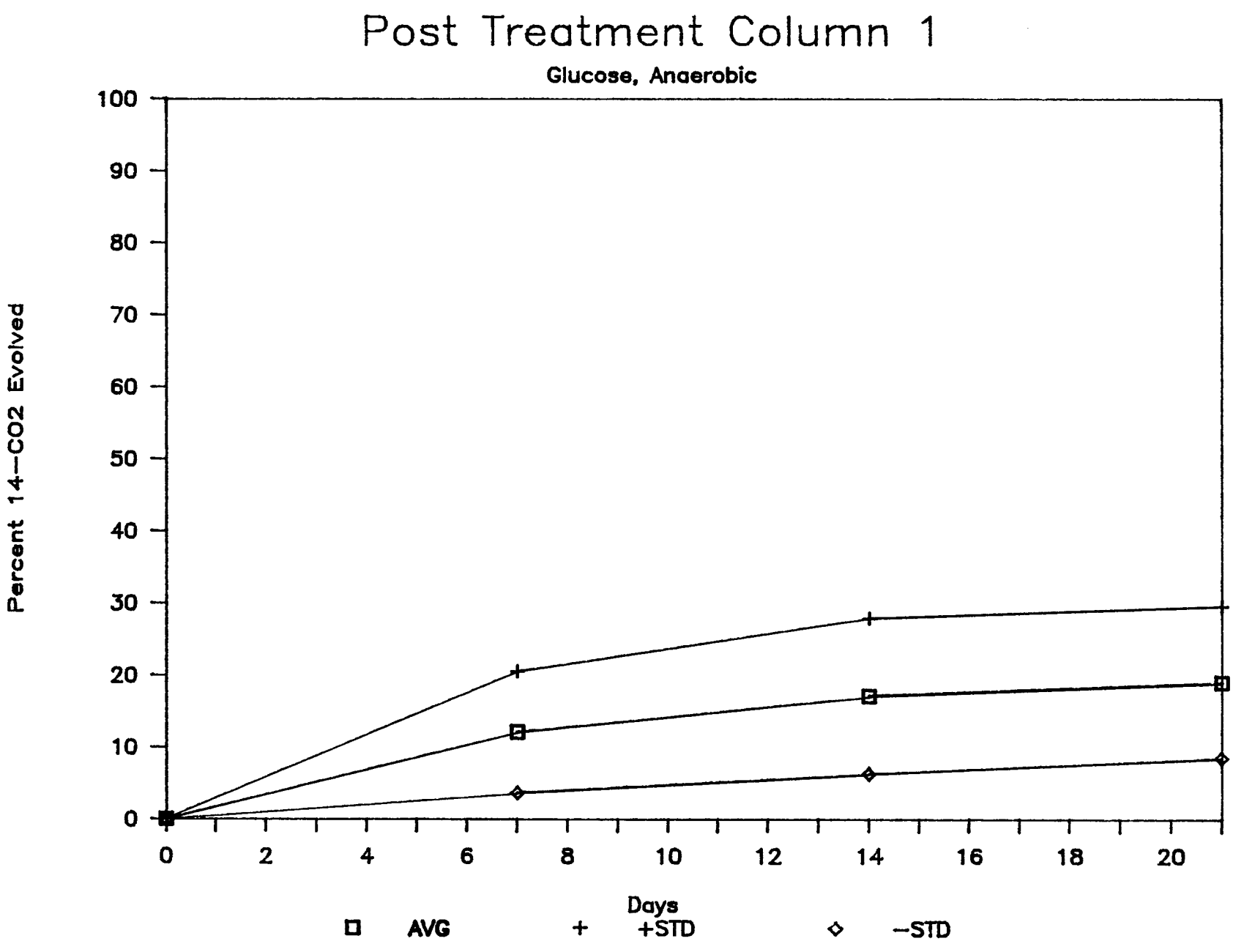

Post Treatment Column 4
Guanidine Nitrate Anaerobic
Percent Evolved 14—CO2
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD


Post Treatment Column 5
Guanidine Nitrate, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

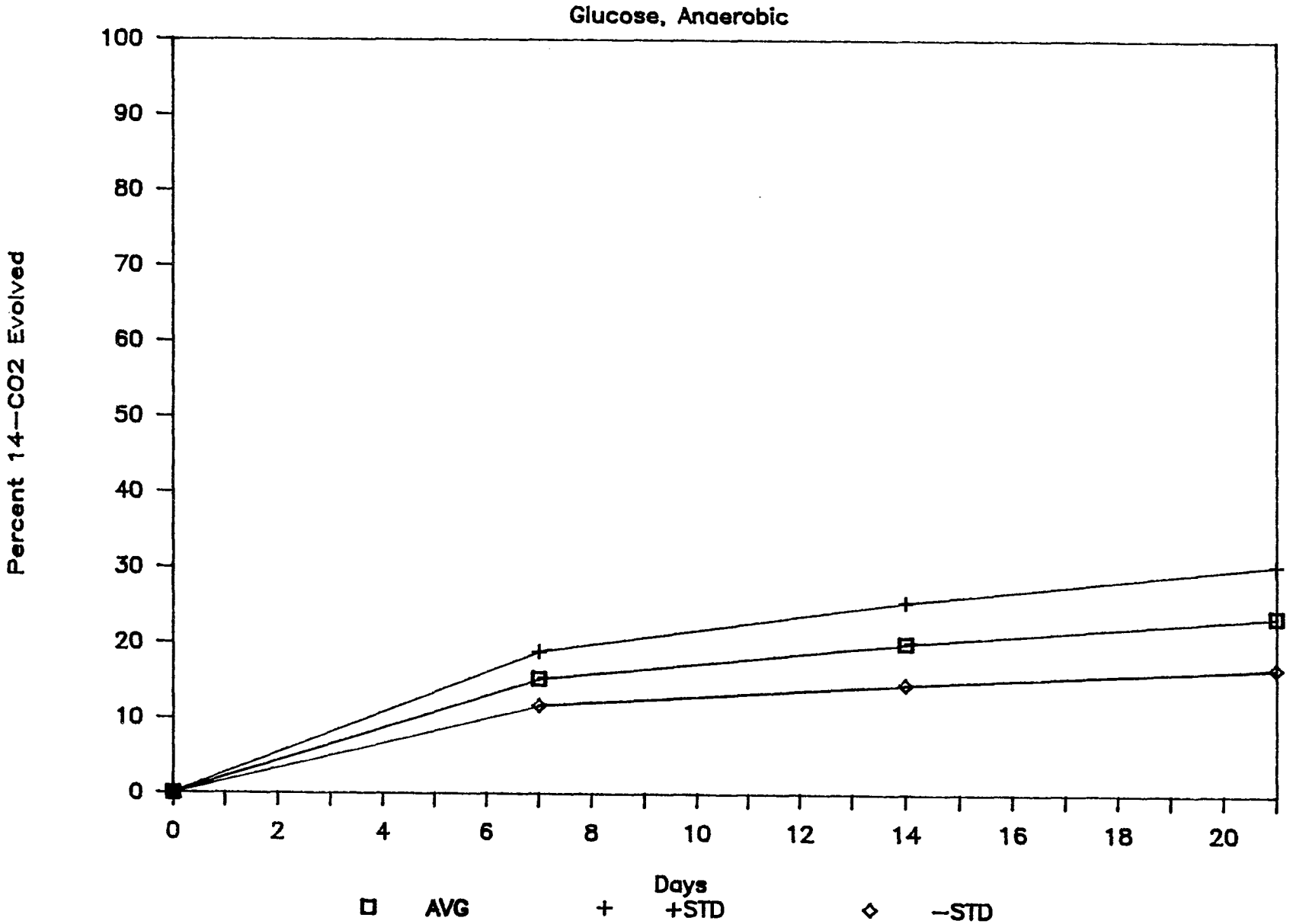

Post Treatment Column 6
Guanidine Nitrate, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

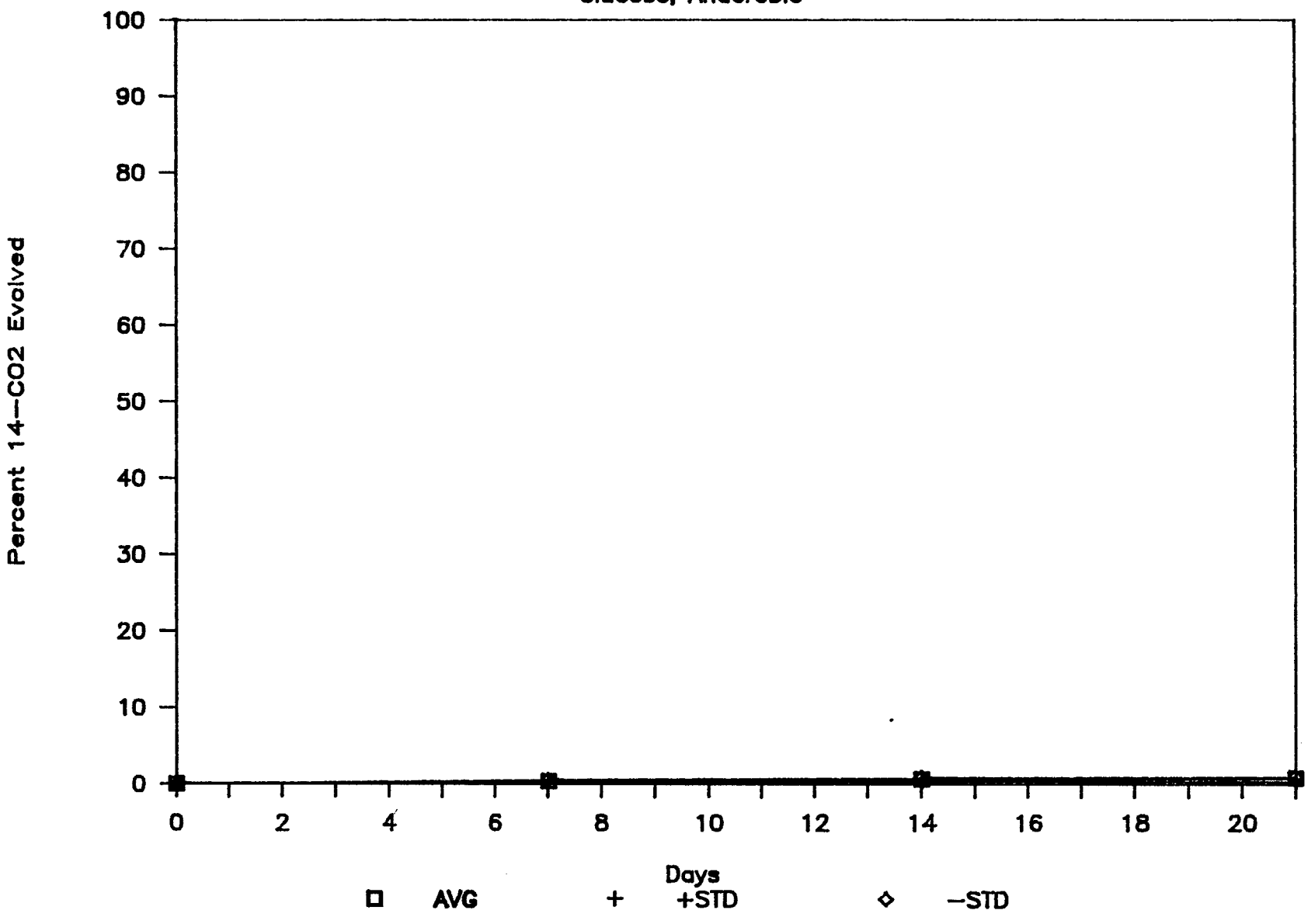
# APPENDIX P

## GRAPHS OF GLUCOSE MINERALIZATION IN POST-TREATMENT SOIL

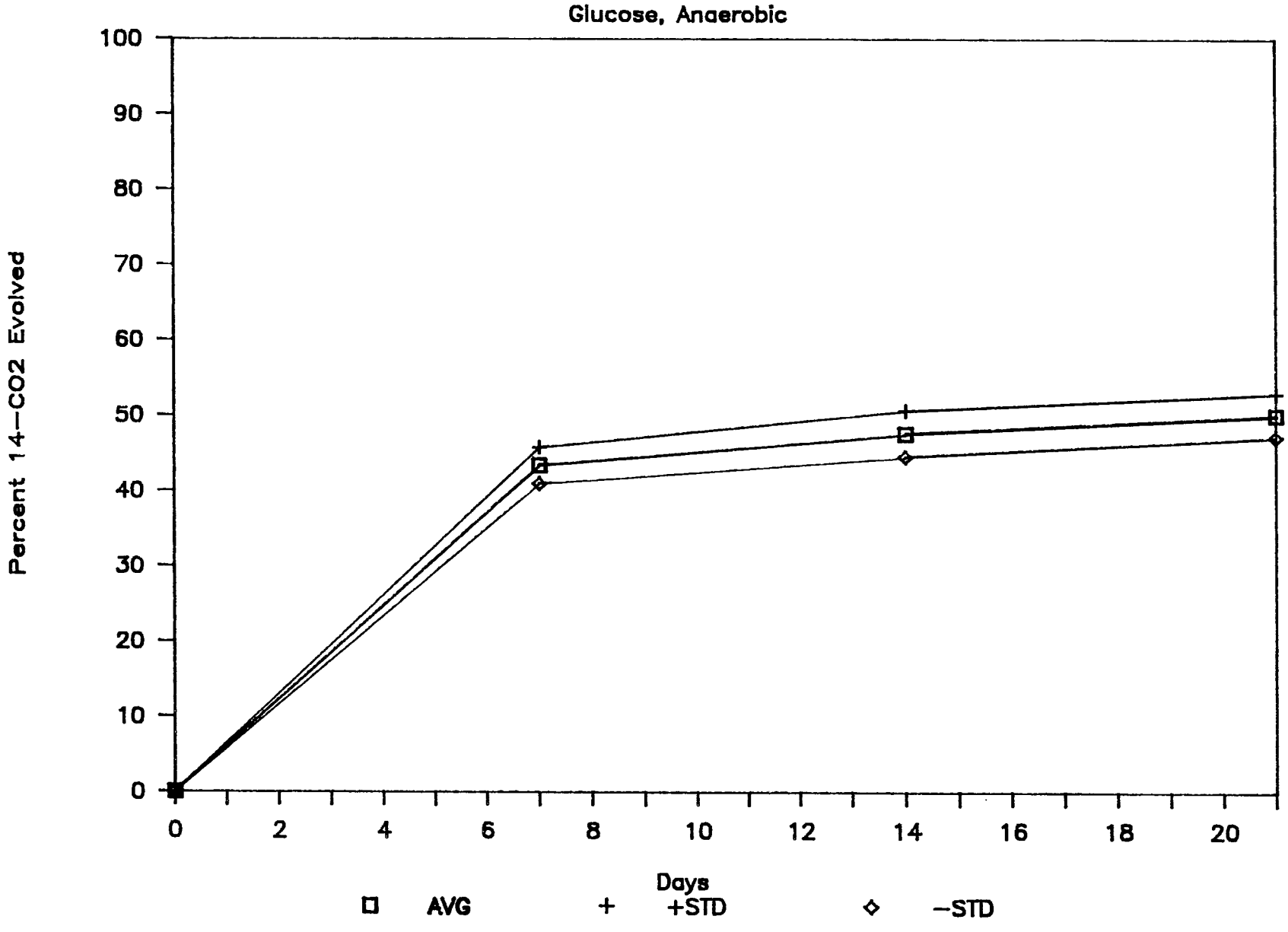

Post Treatment Column 1
Glucose, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

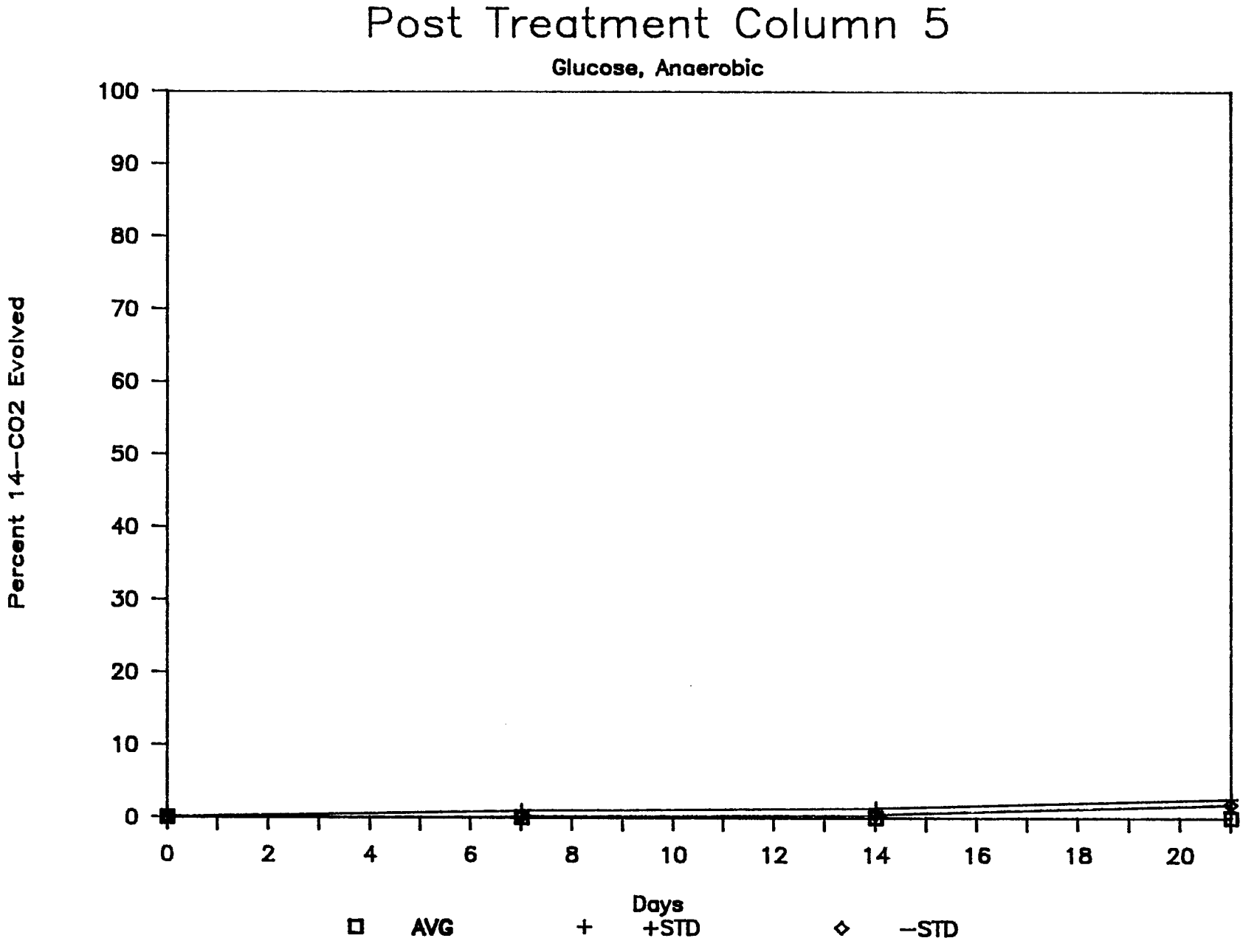
# Post Treatment Column 2



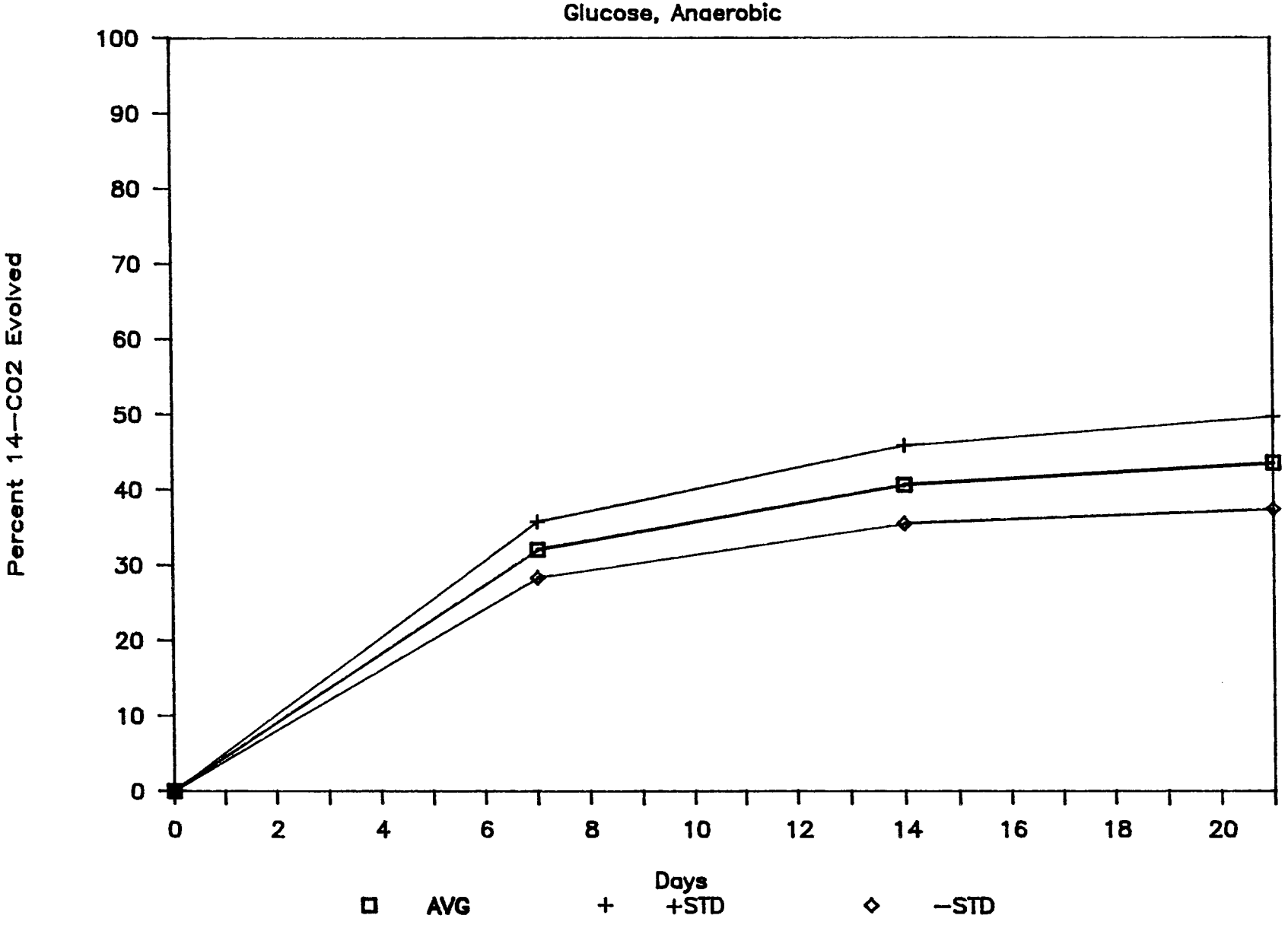

Post Treatment Column 3
Glucose, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

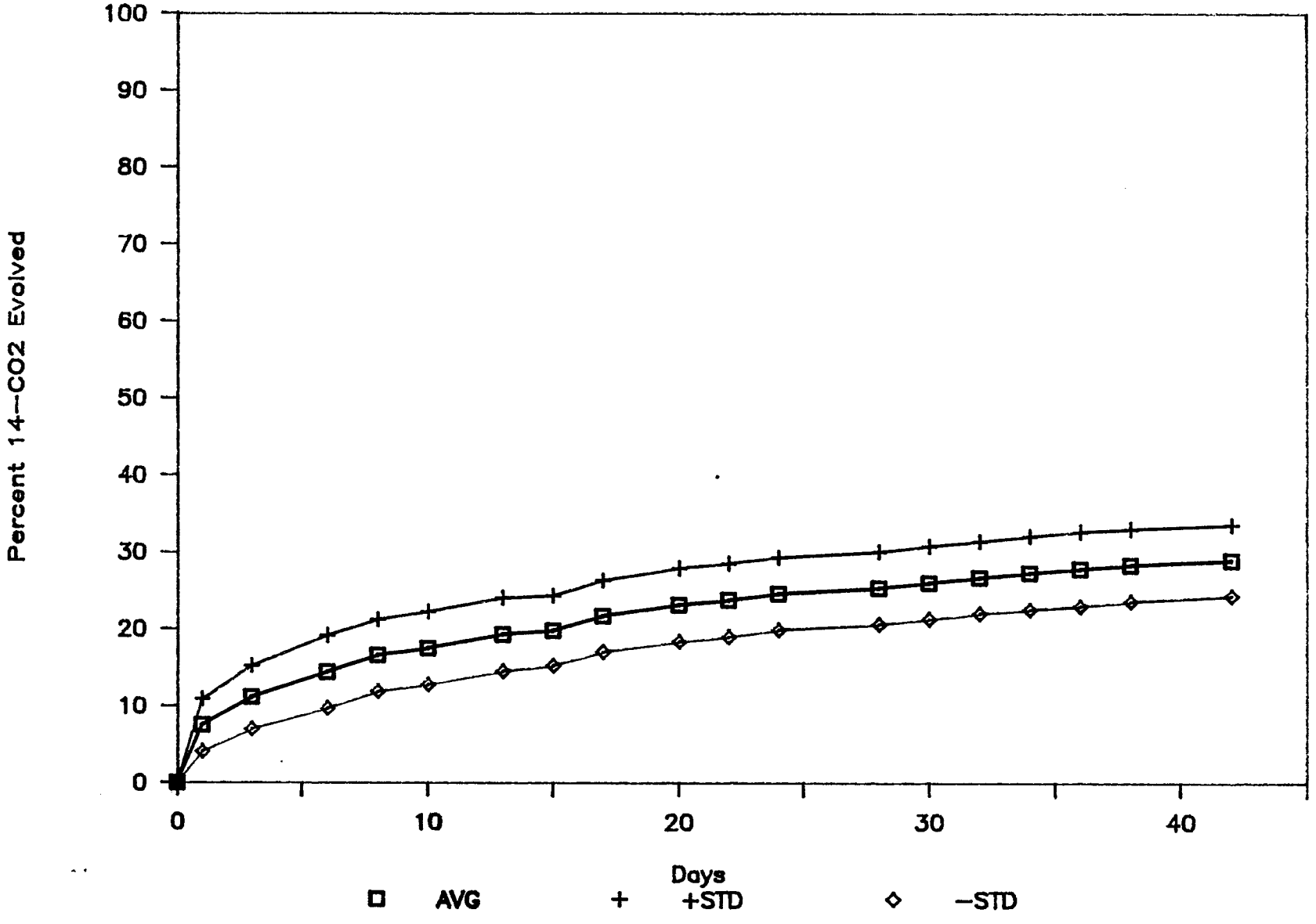
# Post Treatment Column 4



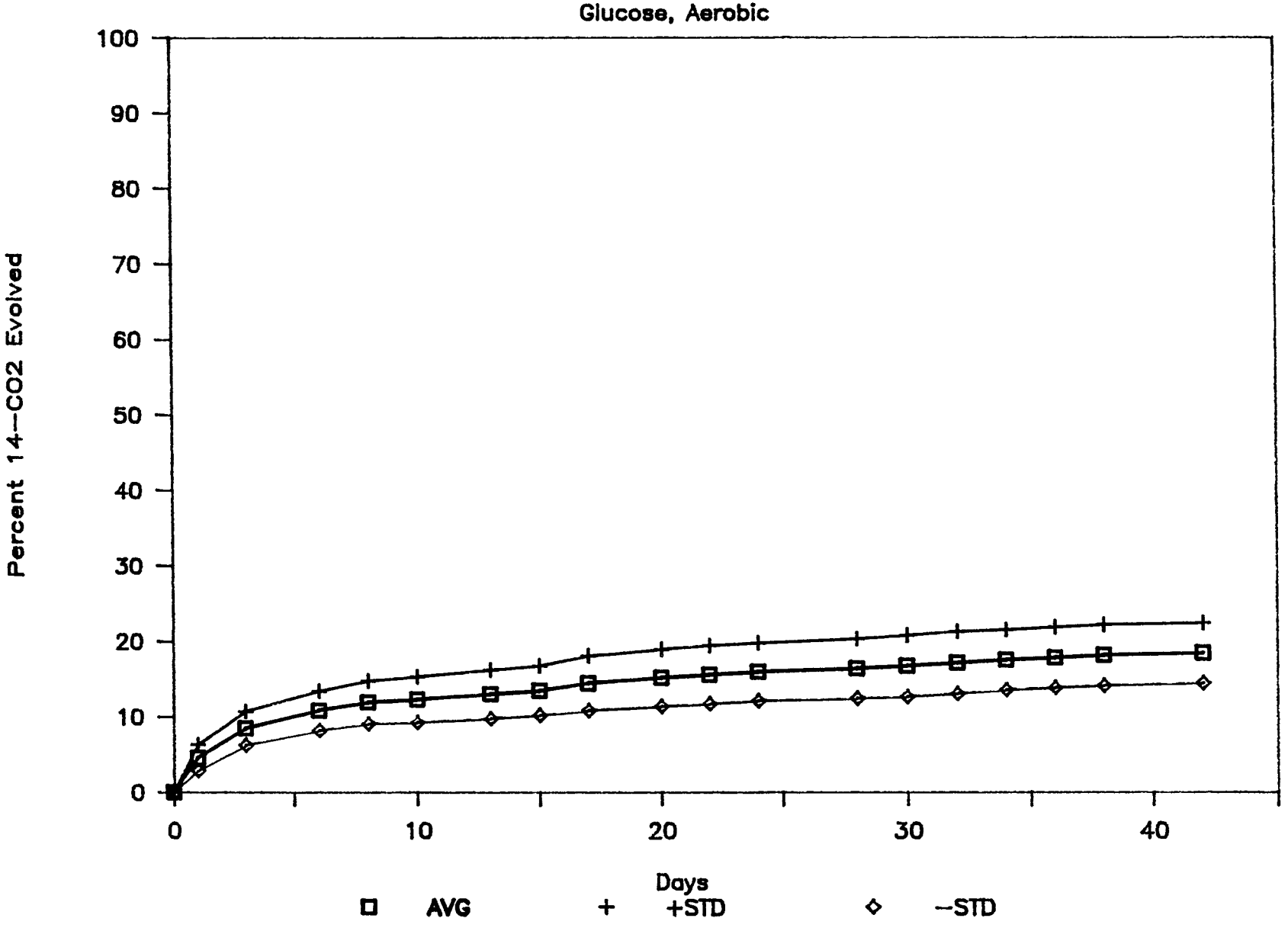

Post Treatment Column 5
Glucose, Anaerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Days
AVG
+STD
—STD

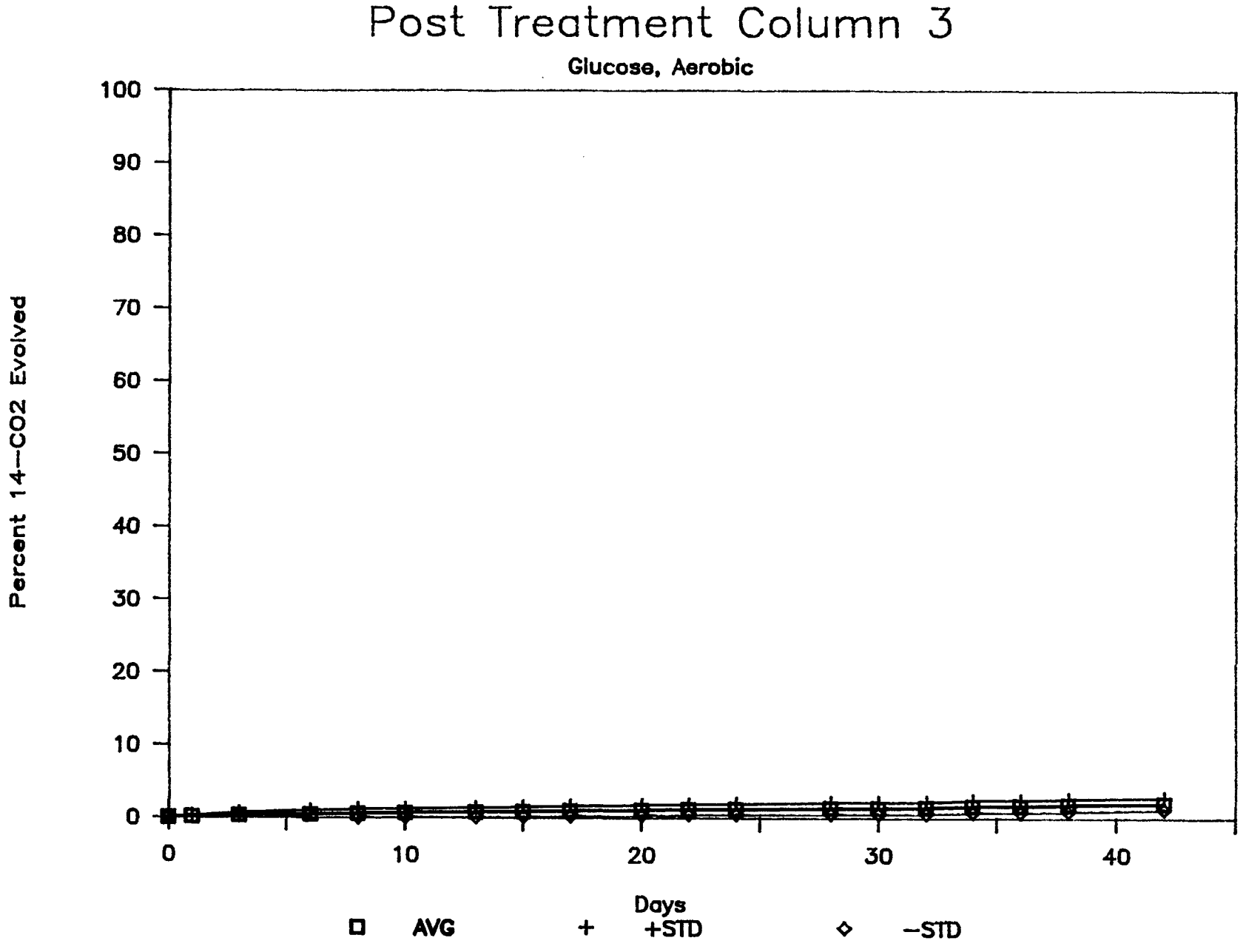
# Post Treatment Column 6



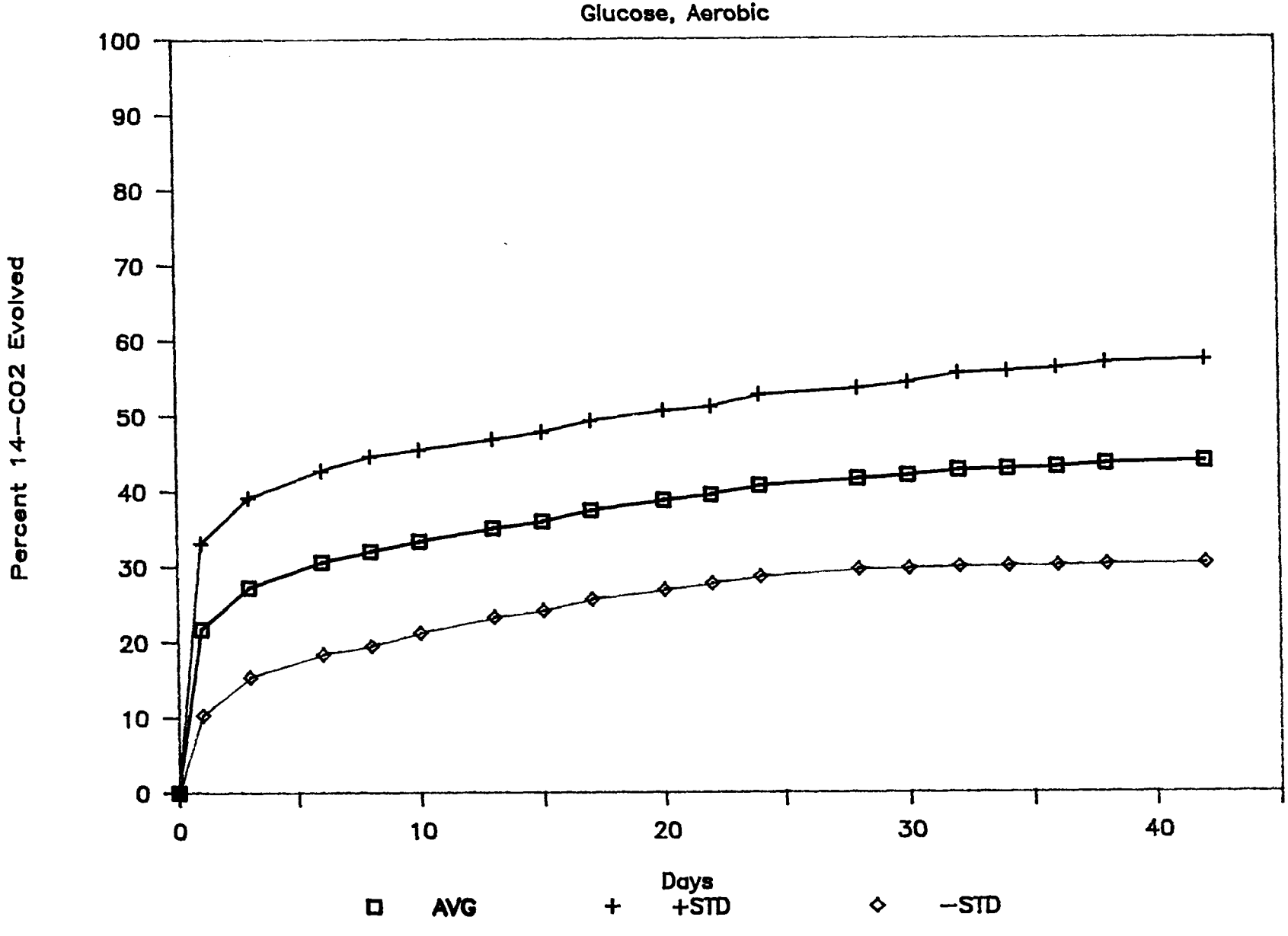

Post Treatment Column 1
Glucose, Aerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
-STD

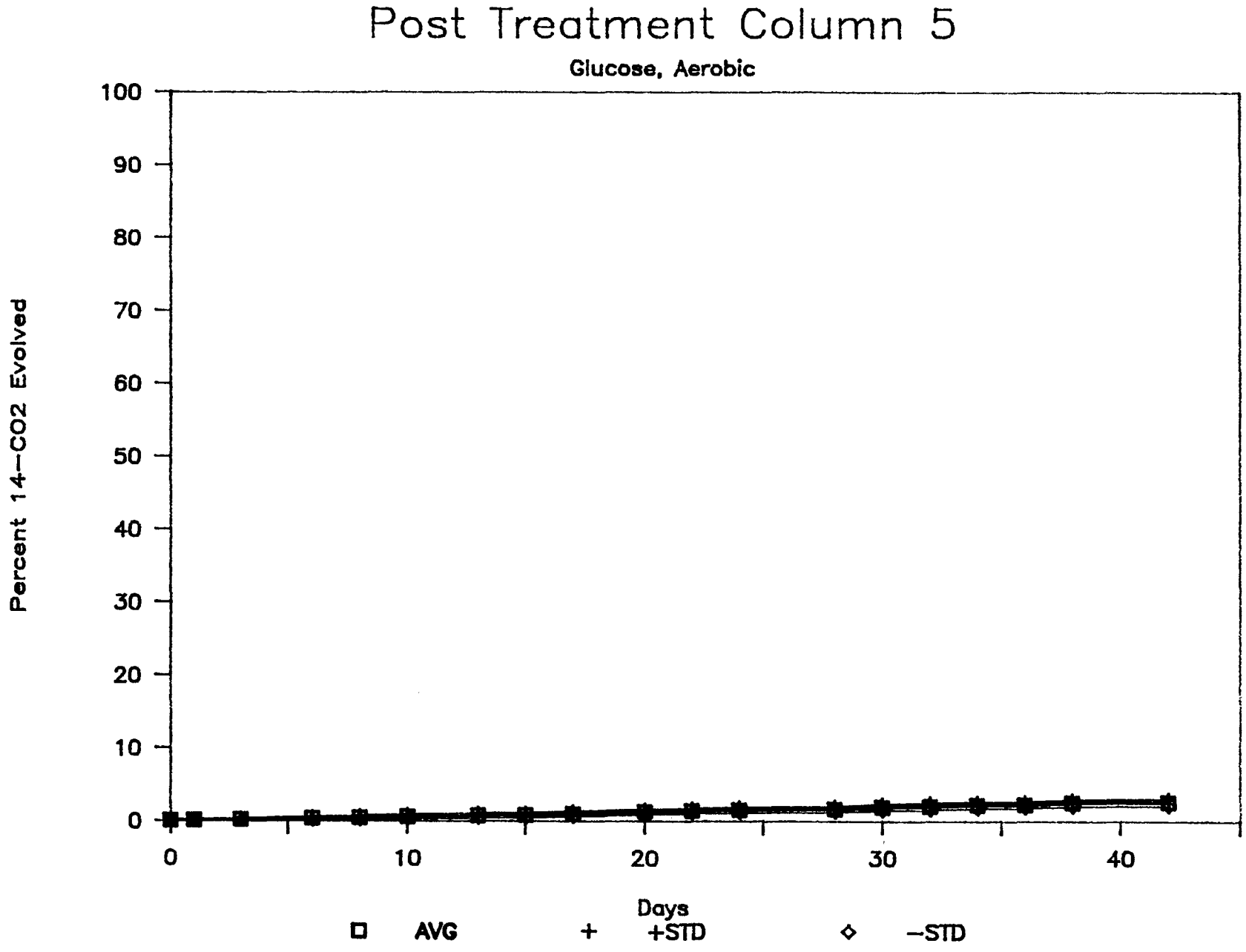
# Post Treatment Column 2

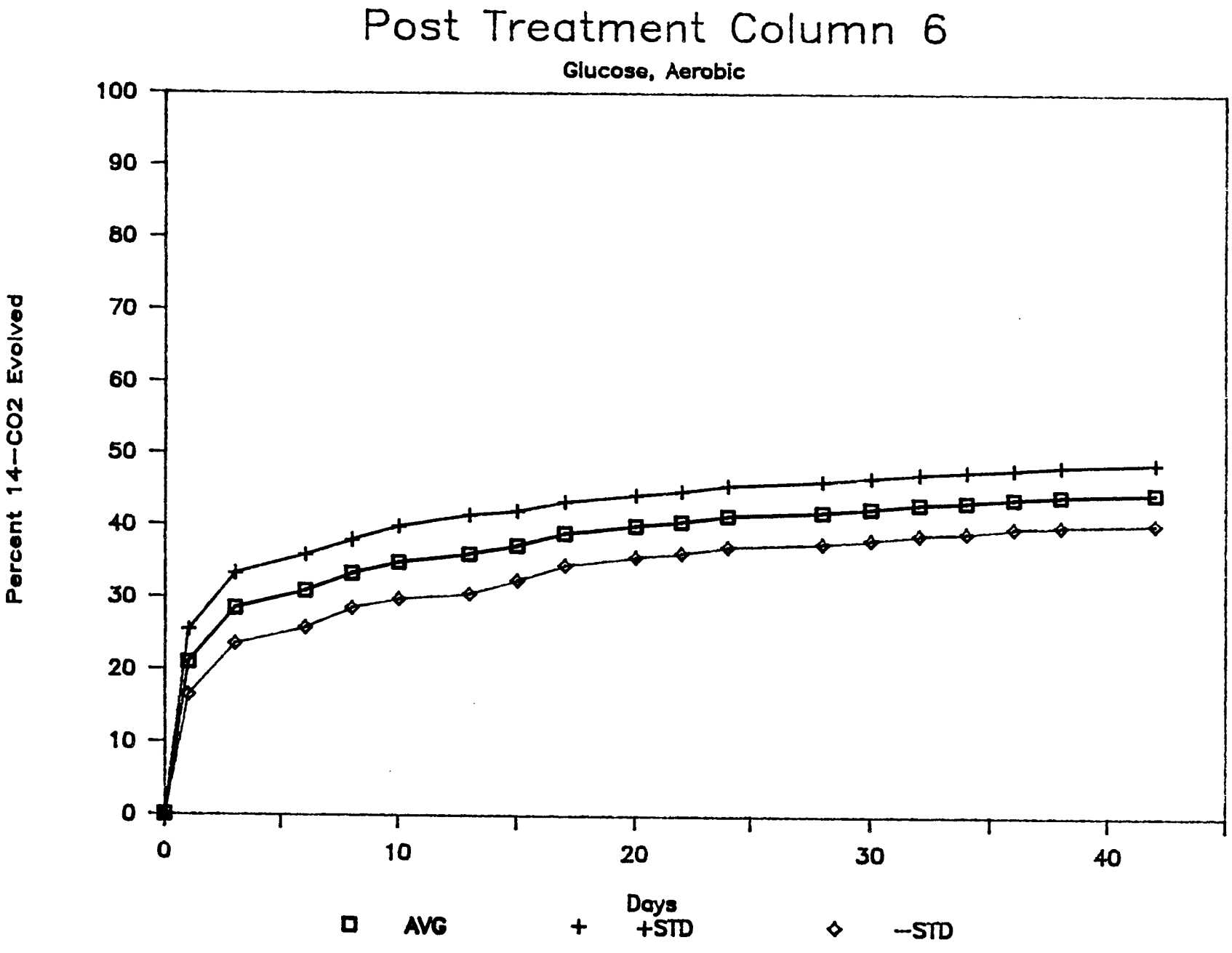



Post Treatment Column 3
Glucose, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD

# Post Treatment Column 4

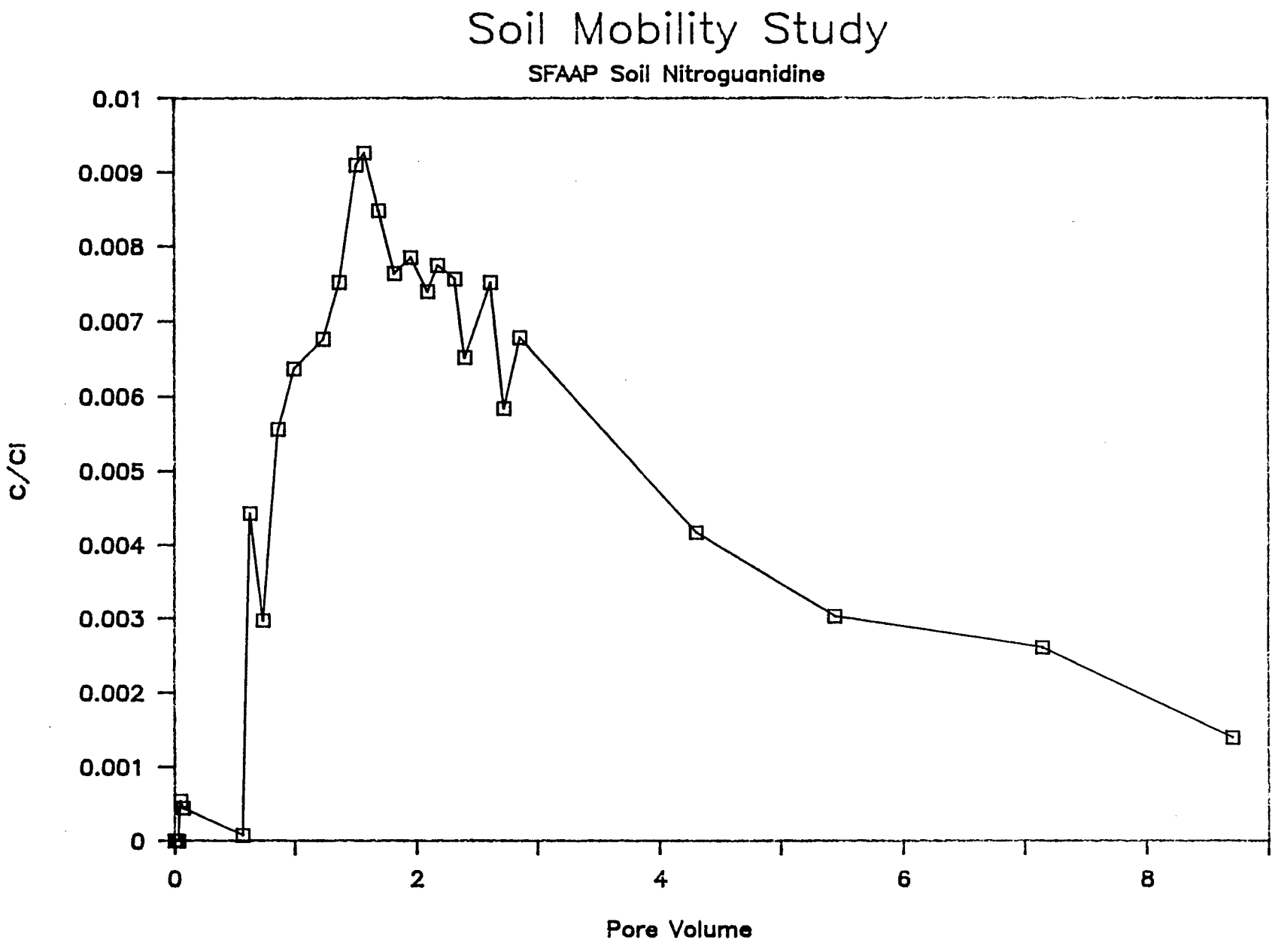



Post Treatment Column 5
Glucose, Aerobic
Percent 14—CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
—STD

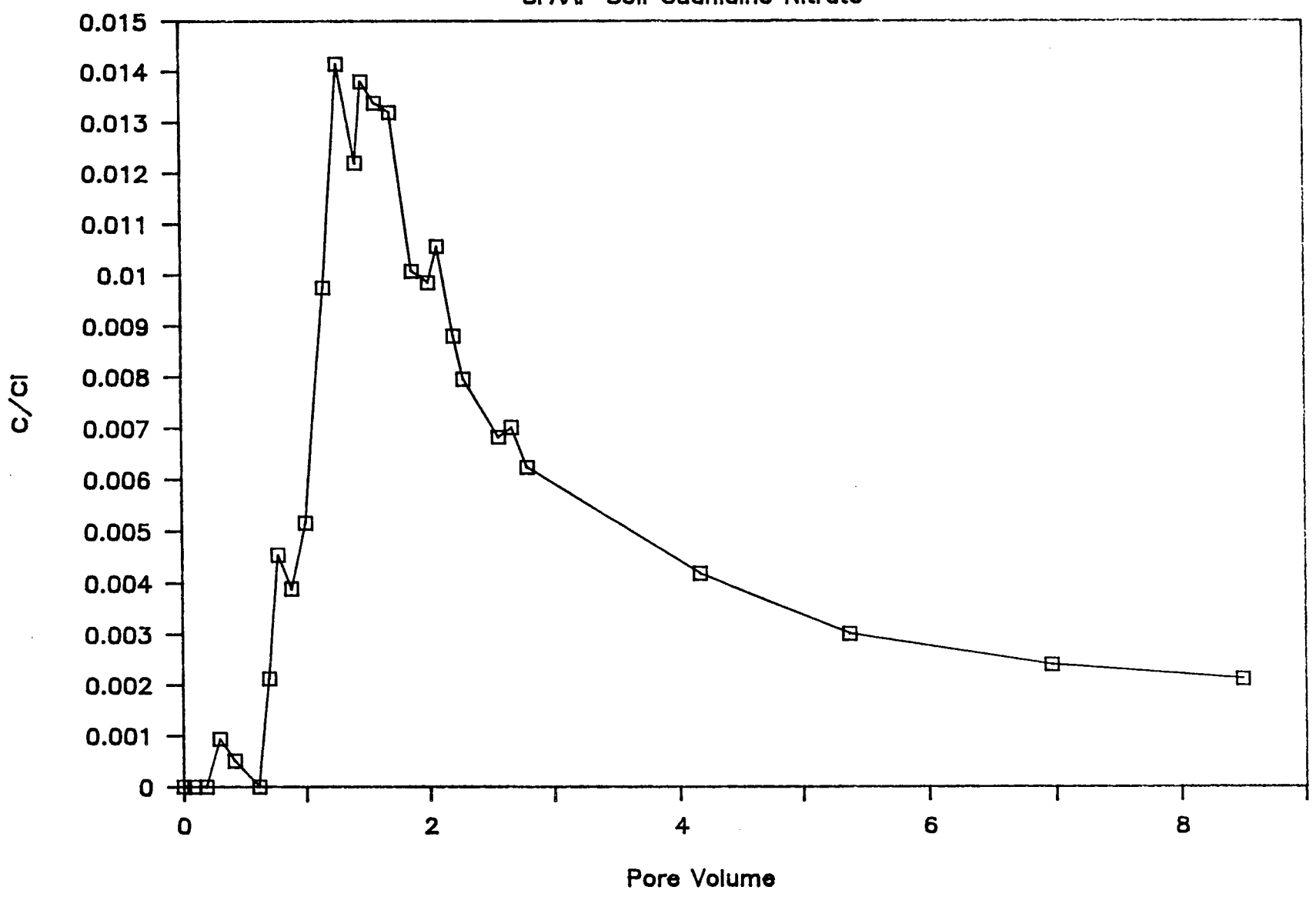

Post Treatment Column 6
Glucose, Aerobic
Percent 14--CO2 Evolved
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Days
AVG
+STD
--STD

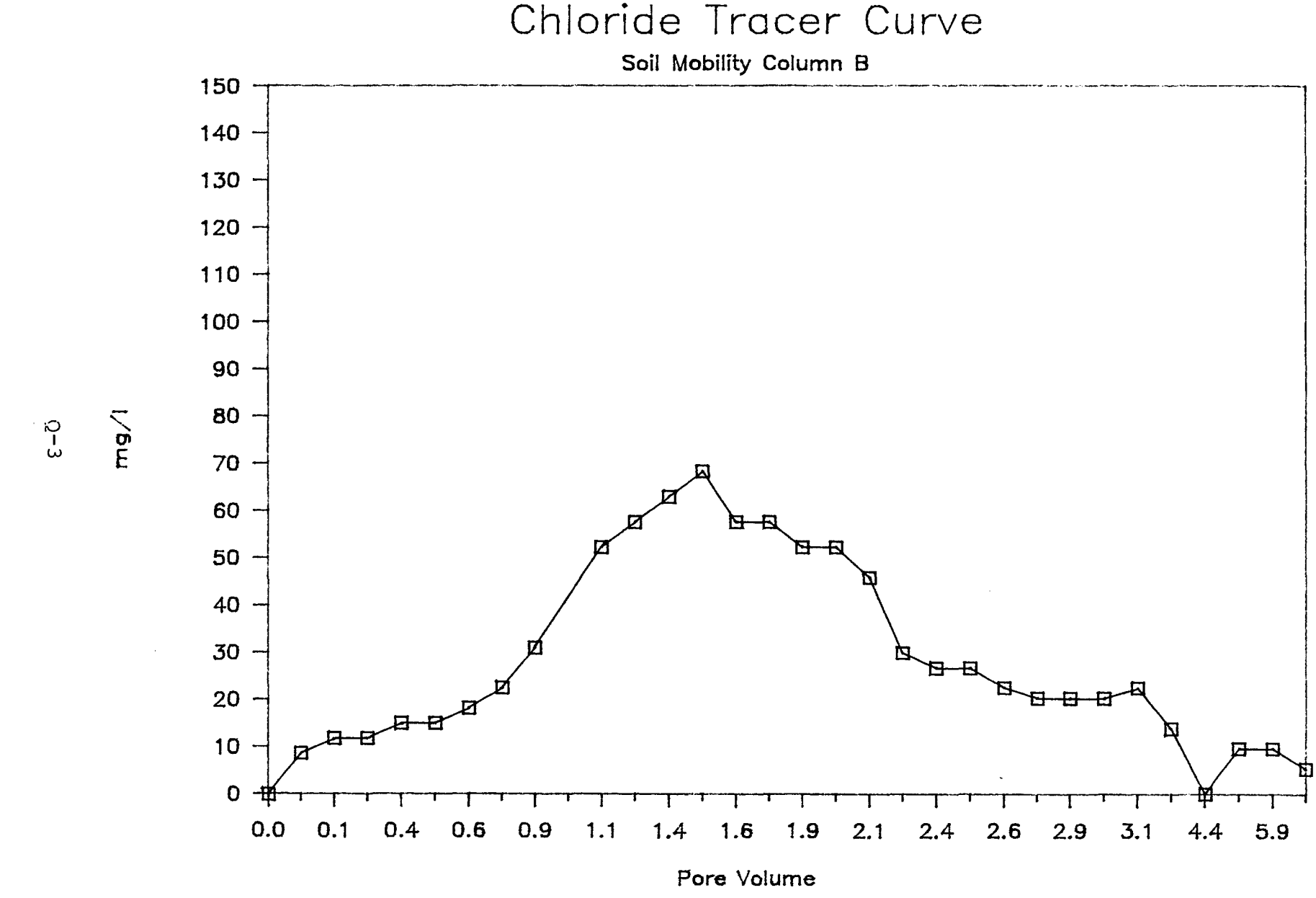
# APPENDIX Q

# NQ AND GN MOBILITY IN SFAAP SOIL


Soil Mobility Study
SFAAP Soil Nitroguanidine
C/Ci
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
2
4
6
8
Pore Volume

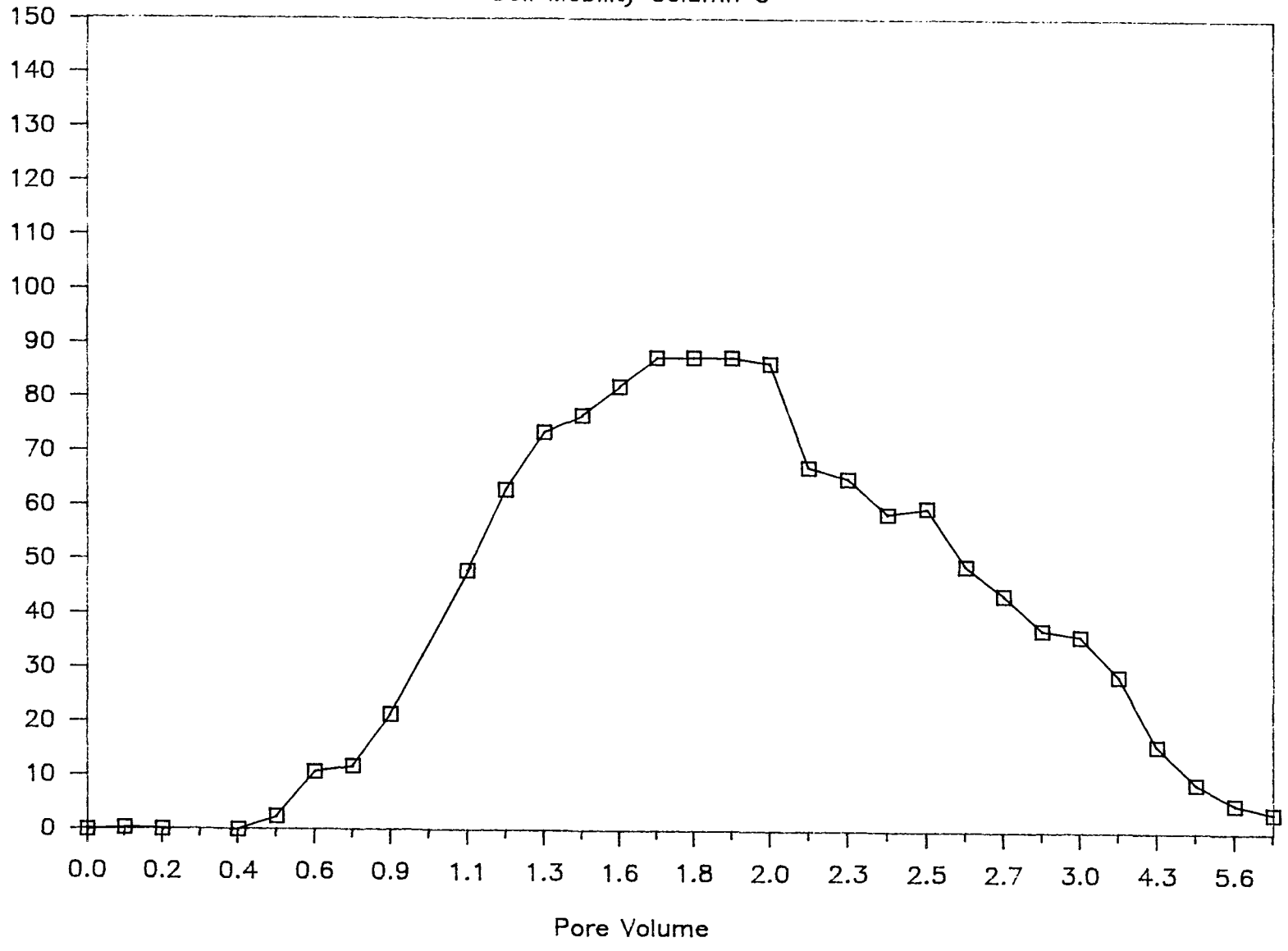
# Soil Mobility Study

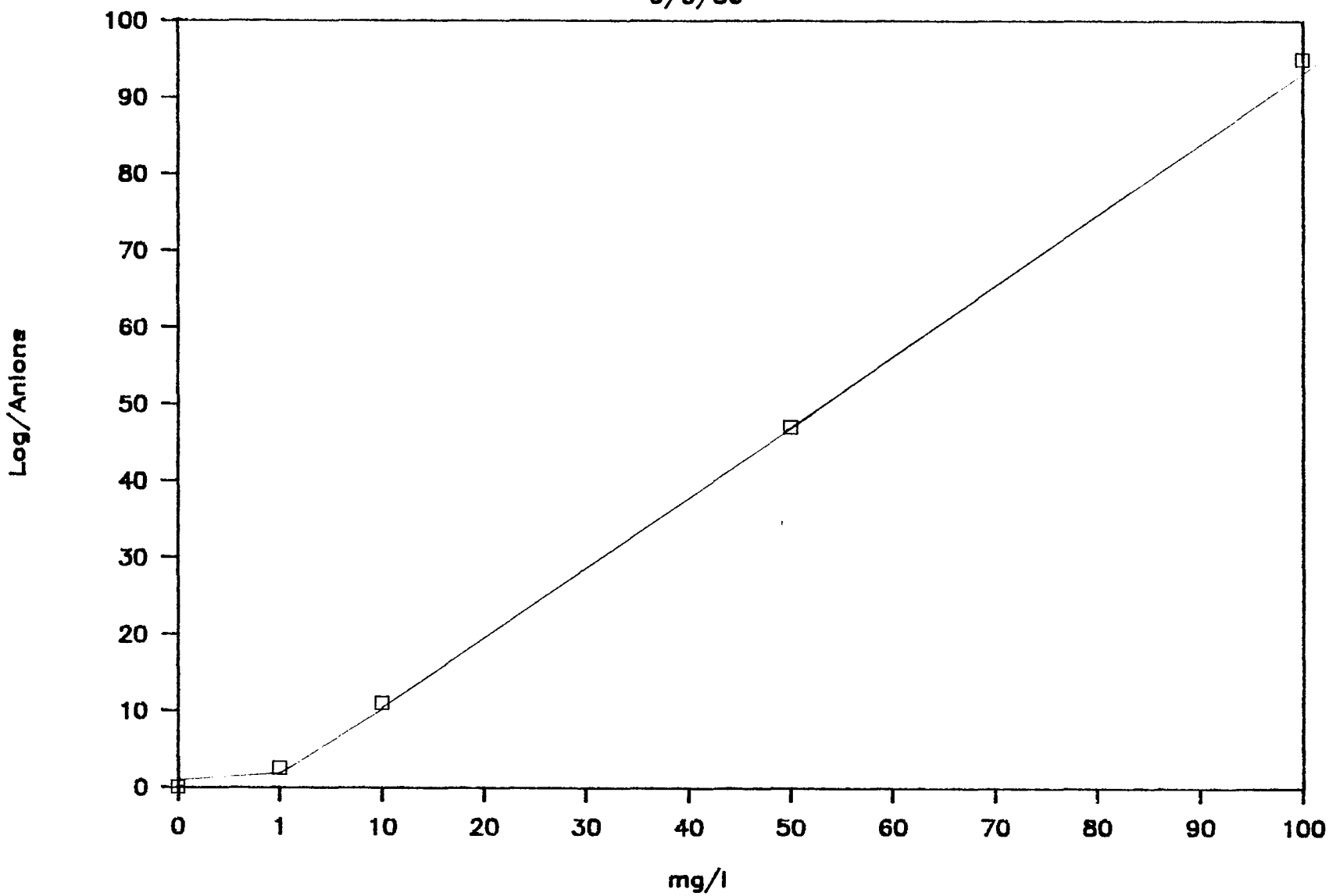



Chloride Tracer Curve
Soil Mobility Column B
mg/l
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0
0.1
0.4
0.6
0.9
1.1
1.4
1.6
1.9
2.1
2.4
2.6
2.9
3.1
4.4
5.9
Pore Volume

# Chloride Tracer Curve

Soil Mobility Column C




Chloride Calibration Curve
9/9/86
Log/Anions
100
90
80
70
60
50
40
30
20
10
0
0
1
10
20
30
40
50
60
70
80
90
100
mg/l

Soil Mobility Study

Nitroguanidine Column

| Time HRS | Initial Conc. dpm/ml | Sample Weight g | | Effluent Volume ml | Pore Volume | Counts dpm/ml | Percent Recovery | C/Ci |
|---|---|---|---|---|---|---|---|---|
| 0 | 30300 | 16.145 | | 0 | 0 | 0 | 0 | 0 |
| 4 | | 18.954 | | 2.809 | 0.017556 | 0.097 | 0.000056 | 0.000003 |
| 8 | | 18.547 | | 2.402 | 0.032568 | 0 | 0.000056 | 0 |
| 12 | | 18.613 | | 2.468 | 0.047993 | 16.4 | 0.008405 | 0.000541 |
| 16 | | 19.777 | | 3.632 | 0.070693 | 13.5 | 0.018518 | 0.000445 |
| 24 | | 219 | 140 | 79 | 0.564443 | 2.2 | 0.054368 | 0.000072 |
| 28 | | 26.441 | | 10.296 | 0.628793 | 134.2 | 0.339377 | 0.004429 |
| 32 | | 33.389 | | 17.244 | 0.736568 | 90 | 0.659501 | 0.002970 |
| 36 | | 35.673 | | 19.528 | 0.858618 | 168.6 | 1.338631 | 0.005564 |
| 40 | | 36.914 | | 20.769 | 0.988425 | 192.8 | 2.164593 | 0.006363 |
| 48 | | 178.4 | | 38.4 | 1.228425 | 204.9 | 3.787563 | 0.006762 |
| 52 | | 37.247 | | 21.102 | 1.360312 | 227.8 | 4.779113 | 0.007518 |
| 56 | | 38.963 | | 22.818 | 1.502925 | 275.6 | 6.076275 | 0.009095 |
| 60 | | 26.672 | | 10.527 | 1.568718 | 280.6 | 6.685573 | 0.009260 |
| 64 | | 34.645 | | 18.5 | 1.684343 | 256.8 | 7.665523 | 0.008475 |
| 68 | | 36.756 | | 20.611 | 1.813162 | 231.3 | 8.648882 | 0.007633 |
| 76 | | 161.8 | | 21.8 | 1.949412 | 237.8 | 9.718197 | 0.007848 |
| 80 | | 38.44 | | 22.295 | 2.088756 | 224.1 | 10.74878 | 0.007396 |
| 84 | | 29.186 | | 13.041 | 2.170262 | 234.6 | 11.37985 | 0.007742 |
| 88 | | 39.102 | | 22.957 | 2.313743 | 229.1 | 12.46472 | 0.007561 |
| 92 | | 29.472 | | 13.327 | 2.397037 | 197.6 | 13.00792 | 0.006521 |
| 90 | | 173.5 | | 33.5 | 2.606412 | 227.8 | 14.58203 | 0.007518 |
| 94 | | 33.788 | | 17.643 | 2.716681 | 177 | 15.22618 | 0.005841 |
| 98 | | 37.156 | | 21.011 | 2.848 | 205.7 | 16.11767 | 0.006788 |
| 153 | | 372 | | 232 | 4.298 | 126.3 | 22.16173 | 0.004168 |
| 196 | | 323.6 | | 183.6 | 5.4455 | 91.9 | 25.64210 | 0.003033 |
| 261 | | 412 | | 272 | 7.1455 | 78.9 | 30.06883 | 0.002603 |
| 321 | | 389 | | 249 | 8.70175 | 42.2 | 32.23628 | 0.001392 |

Soil Mobility Study

Sampling Record
Soil Mobility SFAAP Soil

Column Guanidine Nitrate

| Initial Con'c | Sample Weight g | Effluent Volume ml | Pore Volume | Counts Eff. Con'c dpm/ml | Percent Recovery | C/Ci |
|---|---|---|---|---|---|---|
| 31000 | 16.145 | 0 | 0.0 | 0 | 0 | 0 |
| | 29.54 | 13.395 | 0.1 | 0 | 0 | 0 |
| | 32.03 | 15.885 | 0.2 | 0 | 0 | 0 |
| | 33.956 | 17.811 | 0.3 | 29 | 0.104136 | 0.000935 |
| | 35.09 | 18.945 | 0.4 | 15.6 | 0.163721 | 0.000503 |
| | 171.7 | 31.7 | 0.6 | 0 | 0.163721 | 0 |
| | 29.462 | 13.317 | 0.7 | 66.1 | 0.341192 | 0.002132 |
| | 28.558 | 12.413 | 0.8 | 140.9 | 0.693811 | 0.004545 |
| | 33.373 | 17.228 | 0.9 | 120.7 | 1.113049 | 0.003893 |
| | 35.094 | 18.949 | 1.0 | 160.1 | 1.724689 | 0.005164 |
| | 164.2 | 24.2 | 1.1 | 302.2 | 3.199133 | 0.009748 |
| | 35.208 | 19.063 | 1.3 | 438.7 | 4.885209 | 0.014151 |
| | 38.967 | 22.822 | 1.4 | 378.4 | 6.626307 | 0.012206 |
| | 24.605 | 8.46 | 1.5 | 428 | 7.356323 | 0.013806 |
| | 33.195 | 17.05 | 1.6 | 414.9 | 8.782542 | 0.013383 |
| | 35.481 | 19.336 | 1.7 | 409.1 | 10.37737 | 0.013196 |
| | 167.9 | 27.9 | 1.9 | 312.5 | 12.13518 | 0.010080 |
| | 37.14 | 20.995 | 2.0 | 305.3 | 13.42747 | 0.009848 |
| | 27.783 | 11.638 | 2.1 | 327.5 | 14.19591 | 0.010564 |
| | 37.107 | 20.962 | 2.2 | 273 | 15.34966 | 0.008806 |
| | 28.972 | 12.827 | 2.3 | 246.9 | 15.98817 | 0.007964 |
| | 185 | 45 | 2.6 | 211.5 | 17.90702 | 0.006822 |
| | 32.951 | 16.806 | 2.7 | 217.7 | 18.64465 | 0.007022 |
| | 35.946 | 19.801 | 2.8 | 193.4 | 19.41673 | 0.006238 |
| | 360 | 220 | 4.2 | 129.9 | 25.17843 | 0.004190 |
| | 333.5 | 193.5 | 5.4 | 93.6 | 28.82996 | 0.003019 |
| | 394 | 254 | 7.0 | 74.7 | 32.65532 | 0.002409 |
| | 384 | 244 | 8.5 | 66 | 35.90210 | 0.002129 |

Soil Mobility Column B

| Time HRS | Initial Conc. | Sample Weight | | Effluent Volume | Pore Volume | Log/Anions Reading | | Log/Anions -background | C/Ci | Effluent Concentration | Percent Recovery |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.0 | | | | | 0.0 | |
| 4.0 | 1650.0 | 23.2 | 16.3 | 6.8 | 0.0 | 29.0 | -20 | 9.0 | 0.0 | 8.7 | 0.2 |
| 8.0 | NaCl(mg/l) | 31.1 | | 14.8 | 0.1 | 32.0 | | 12.0 | 0.0 | 11.9 | 0.9 |
| 12.0 | | 38.6 | | 22.3 | 0.3 | 32.0 | | 12.0 | 0.0 | 11.9 | 1.9 |
| 16.0 | | 34.0 | | 17.7 | 0.4 | 35.0 | | 15.0 | 0.0 | 15.1 | 2.9 |
| 20.0 | | 37.2 | | 20.9 | 0.5 | 35.0 | | 15.0 | 0.0 | 15.1 | 4.1 |
| 24.0 | | 35.1 | | 18.8 | 0.6 | 38.0 | | 18.0 | 0.0 | 18.3 | 5.4 |
| 28.0 | | 35.1 | | 18.8 | 0.8 | 42.0 | | 22.0 | 0.0 | 22.6 | 7.0 |
| 32.0 | | 36.4 | | 20.1 | 0.9 | 50.0 | | 30.0 | 0.0 | 31.1 | 9.2 |
| 36.0 | | 36.4 | | 20.1 | 1.0 | | | | 0.0 | | 9.2 |
| 40.0 | | 36.5 | | 20.2 | 1.1 | 70.0 | | 50.0 | 0.0 | 52.4 | 13.1 |
| 44.0 | | 36.8 | | 20.5 | 1.3 | 75.0 | | 55.0 | 0.0 | 57.7 | 17.3 |
| 48.0 | | 35.3 | | 19.0 | 1.4 | 80.0 | | 60.0 | 0.0 | 63.1 | 21.7 |
| 52.0 | | 36.2 | | 19.9 | 1.5 | 85.0 | | 65.0 | 0.0 | 68.4 | 26.6 |
| 56.0 | | 37.5 | | 21.2 | 1.6 | 75.0 | | 55.0 | 0.0 | 57.7 | 31.0 |
| 60.0 | | 34.9 | | 18.6 | 1.7 | 75.0 | | 55.0 | 0.0 | 57.7 | 34.9 |
| 64.0 | | 35.6 | | 19.3 | 1.9 | 70.0 | | 50.0 | 0.0 | 52.4 | 38.5 |
| 68.0 | | 37.3 | | 21.0 | 2.0 | 70.0 | | 50.0 | 0.0 | 52.4 | 42.5 |
| 72.0 | | 36.9 | | 20.6 | 2.1 | 64.0 | | 44.0 | 0.0 | 46.0 | 45.9 |
| 76.0 | | 37.9 | | 21.5 | 2.3 | 49.0 | | 29.0 | 0.0 | 30.0 | 48.3 |
| 80.0 | | 34.5 | | 18.2 | 2.4 | 46.0 | | 26.0 | 0.0 | 26.8 | 50.1 |
| 84.0 | | 30.8 | | 14.5 | 2.5 | 46.0 | | 26.0 | 0.0 | 26.8 | 51.5 |
| 88.0 | | 37.9 | | 21.6 | 2.6 | 42.0 | | 22.0 | 0.0 | 22.6 | 53.3 |
| 92.0 | | 38.4 | | 22.1 | 2.7 | 40.0 | | 20.0 | 0.0 | 20.4 | 55.0 |
| 96.0 | | 35.6 | | 19.2 | 2.9 | 40.0 | | 20.0 | 0.0 | 20.4 | 56.4 |
| 100.0 | | 40.7 | | 24.4 | 3.0 | 40.0 | | 20.0 | 0.0 | 20.4 | 58.3 |
| 104.0 | | 31.3 | | 15.0 | 3.1 | 42.0 | | 22.0 | 0.0 | 22.6 | 59.5 |
| 128.0 | | 229.5 | 140.0 | 89.5 | 3.7 | 34.0 | | 14.0 | 0.0 | 14.0 | 64.3 |
| 152.0 | | 253.0 | | 113.0 | 4.4 | 21.0 | | 1.0 | 0.0 | 0.2 | 64.7 |
| 176.0 | | 256.5 | | 116.5 | 5.1 | 30.0 | | 10.0 | 0.0 | 9.8 | 69.1 |
| 200.0 | | 273.5 | | 133.5 | 5.9 | 30.0 | | 10.0 | 0.0 | 9.8 | 74.2 |
| 224.0 | | 168.0 | | 28.0 | 6.1 | 26.0 | | 6.0 | 0.0 | 5.5 | 74.8 |

Soil Mobility Test
Chloride Tracer Study

Column C

| Time HRS | Initial Con'c NaCl(mg/l | Sample Weight g | | Effluent Volume ml | Pore Volume | Log/Anions Reading | -background | Effluent Con'c | Percent Recovery |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.0 | 0 | | 0.0 | |
| 4 | 1650 | 28.746 | 16.3 | 12.446 | 0.1 | 3.2 | 1.2 | 0.4 | 0.017746 |
| 8 | | 31.006 | | 14.706 | 0.2 | 2.9 | 0.9 | 0.1 | 0.020896 |
| 12 | | 36.656 | | 20.356 | 0.3 | 2.5 | 0.5 | -0.4 | -0.00763 |
| 16 | | 31.586 | | 15.286 | 0.4 | 2.9 | 0.9 | 0.1 | -0.00435 |
| 20 | | 35.866 | | 19.566 | 0.5 | 5.2 | 3.2 | 2.5 | 0.181600 |
| 24 | | 37.501 | | 21.201 | 0.6 | 13 | 11 | 10.8 | 1.051046 |
| 28 | | 33.462 | | 17.162 | 0.8 | 14 | 12 | 11.9 | 1.824174 |
| 32 | | 35.492 | | 19.192 | 0.9 | 23 | 21 | 21.5 | 3.386427 |
| 36 | | 34.509 | | 18.209 | 1.0 | | | | 3.386427 |
| 40 | | 35.165 | | 18.865 | 1.1 | 48 | 46 | 48.1 | 6.827030 |
| 44 | | 35.624 | | 19.324 | 1.2 | 62 | 60 | 63.1 | 11.44408 |
| 48 | | 34.119 | | 17.819 | 1.3 | 72 | 70 | 73.7 | 16.42128 |
| 52 | | 34.967 | | 18.667 | 1.5 | 75 | 73 | 76.9 | 21.86155 |
| 56 | | 35.187 | | 18.887 | 1.6 | 80 | 78 | 82.3 | 27.74737 |
| 60 | | 33.799 | | 17.499 | 1.7 | 85 | 83 | 87.6 | 33.55404 |
| 64 | | 34.2 | | 17.9 | 1.8 | 85 | 83 | 87.6 | 39.49378 |
| 68 | | 35.944 | | 19.644 | 1.9 | 85 | 83 | 87.6 | 46.01224 |
| 72 | | 36.199 | | 19.899 | 2.0 | 84 | 82 | 86.5 | 52.53493 |
| 76 | | 36.792 | | 20.492 | 2.2 | 66 | 64 | 67.3 | 57.76213 |
| 80 | | 34.359 | | 18.059 | 2.3 | 64 | 62 | 65.2 | 62.22283 |
| 84 | | 30.61 | | 14.31 | 2.4 | 58 | 56 | 58.8 | 65.41069 |
| 88 | | 36.411 | | 20.111 | 2.5 | 59 | 57 | 59.9 | 69.97209 |
| 92 | | 37.003 | | 20.703 | 2.6 | 49 | 47 | 49.2 | 73.83153 |
| 96 | | 34.609 | | 18.309 | 2.7 | 44 | 42 | 43.9 | 76.87492 |
| 100 | | 38.48 | | 22.18 | 2.9 | 38 | 36 | 37.5 | 80.02422 |
| 104 | | 30.627 | | 14.327 | 3.0 | 37 | 35 | 36.4 | 82.00063 |
| 128 | | 229.8 | 140 | 89.8 | 3.5 | 30 | 28 | 29.0 | 91.84947 |
| 152 | | 266 | | 126 | 4.3 | 18 | 16 | 16.2 | 99.56136 |
| 176 | | 246 | | 106 | 5.0 | 11.5 | 9.5 | 9.2 | 103.2661 |
| 200 | | 243.8 | | 103.8 | 5.6 | 8 | 6 | 5.5 | 105.4266 |
| 204 | | 181 | | 41 | 5.9 | 6.4 | 4.4 | 3.8 | 106.0150 |